AD NUMBER

AD846830

NEW LIMITATION CHANGE

TO

Approved for public release, distribution unlimited

FROM

Distribution authorized to U.S. Gov't. agencies and their contractors; Administrative/Operational Use; 15 DEC 1964. Other requests shall be referred to Department of the Army, Fort Detrick, MD.

AUTHORITY

SMUFD D/A ltr, 14 Feb 1972

AD NUMBER

AD846830

NEW LIMITATION CHANGE

TO

Distribution authorized to U.S. Gov't. agencies and their contractors; Administrative/Operational Use; 15 DEC 1964. Other requests shall be referred to Department of the Army, Fort Detrick, MD.

FROM

Approved for public release, distribution unlimited

AUTHORITY

SMUFD D/A ltr, 14 Feb 1972 xxxxxx

TRANSLATION NO. 1251

DATE: 15 Dec 1964

DDC AVAILABILITY NOTICE

This document is subject to special export controls and each transmittal to foreign governments or foreign nationals may be made only with prior approval of Commanding Officer, Fort Detrick, ATTN: SMUFD-AE-T, Frederick, Md. 21701.

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

#1251

CLOSTRIDIUM BOTULINUM AS A PROBLEM IN FOOD HANDLING

Report No 100, 1961, pages 1-108
on a symposium held 15 February 1961 by the
Swedish Institute for Food Preservation Research, Goteborg

CONTENTS

INTRODUCTION

At a meeting in Malmoe on 12 March 1960, arranged by the Swedish Institute for People's Health (Statens Institut for Folkhälsan), bacteriological control of deep-freezed articles was discussed. Possible risks of development of aneorobic micro-organisms, especially Cl. botulinum in vacuum-packed articles, were also discussed.

At the invitation of Aekerlund & Rausing, Ltd., a preparative symposium was arranged in June of 1960 in order to discuss whether vacuum-packing means increased risk of botulism.

The participants of that symposium considered it desirable that the work be continued and be given a broader basis and that another symposium be arranged in which representatives from our Nordic neighbor countries would participate after the results of new investigations had appeared. Such a symposium was arranged in Goteborg on 15 February 1961 at the Swedish Institute for Food Preservation Research under the chairmanship of Prof. Orjan Ouchterlony of the Goteborg University.

At the request of the participants, the collected speeches are herewith edited. The speech by A. Johannsen, City Veterinarian, was part of the investigation that he has made at the request and cost of Aekerlund & Rausing, Ltd, and the investigation in its entirety has been placed at our disposal for publication through their kind cooperation.

Goeteborg, 1 May 1961

Erik von Sydow

CLOSTRIDIUM BOTULINUM

A Literature Study by Nils Molin

Clostridium botulinum was first isolated by Ermengen in 1895 in connection with an investigation of the reason for a case of food poisoning. The appellation is the same for several types of bacteria, which all produce toxin with similar pharmacological effects.

The antigenic characteristics of the toxins form the basis of their classification. The following types have been isolated: Cl. botulinum, types A, B, C, D, and E. Type C is further divided into two sub-groups. Antitoxin produced of one type does not neutralize toxin of another type, with the exception of C and D which to some extent seem to be able to replace each other. Biochemically there are two main groups. The one is proteolytic in its nature, the other saccharolytic.

Cl. botulinum is a rod bacterium that produces spores and is strictly anaeorobic. Young cultures are gram positive. Boroff and his collaborator found in 1952 that Cl. botulinum, type E, quickly lost its gram positive quality, in which process the bacteria became thinner and thinner and finally came apart in gram negative rods. Like most other anaeorobes Cl. botulinum can grow in the presence of oxygen if the red-ox potential in the medium is sufficiently low. According to Dack and Willisten in 1929 the vitality was considerably decreased in young cultures of type A and B when these were exposed to oxygen. The age of the culture and the composition of the medium turned out to affect the resistance of the cells. The non-proteolytic rods are more sensitive to oxygen than the proteolytic ones, according to Gunnison and Meyer, 1929.

Cl. botulinum makes great demands upon the composition of the nutrient medium. It is considered that most rods demand the amino acids: L-alanine, L-phenylalanine, and arginine. The growth is stimulated by, among other things, glycine, ornithine, creatine, and sodium valeric. In 1929, Gunnison and Meyer investigated 53 rods of both American and European origin with

regard to their proteolytic and peptolytic qualities. They found that all investigated A-rods and all American B-rods could attack protein and its broken down components. The European B-rods varied in proteolytic activity, and types C, D, and E were on the whole not capable breaking down protein. Gunnison and Meyer state that when the non-proteolytic rods grow in food they only give rise to an acid smell which reminds of lactic acid fermentation, while the presence of growing proteolytic rods produces a typical rotten smell. Most rods in Gunnison and Meyer's material were saccharolytic and could break up glucose, fructose, and maltose. Proteolytic rods are at times called Cl. parabotulinum.

The botulinum toxins resemble each other pharmacologically in that they all give rise to botulism. The toxin of types A, B, and E, as well as certain C rods, attack human beings. Botulism in minks has been associated with toxins of types A and C. Types B and C have been isolated in affected birds, and types C and D in ruminants.

It was formerly thought that the toxin is a product of the metabolism of the bacteria and secreted in the surrounding substrate of living Clostridia. Later, scientists have shown that the botulinus toxin is not a true secretion, but rather an autolytic product, basing this view upon observations that young cultures in the logarithmic phase of growth do not produce maximal amounts of toxin. In experiments in 1959 and 1960 by Bonventre and Kempe the toxitivity of the extracellular and intracellular toxin at the end of the logarithmic phase of growth was only 10% of what could be demonstrated when the autolysis was complete. The authors believe that during the active growth a protoxin is produced which later, when the cells are autolyzed, is activated and freed with cooperation of proteolytic enzymes. Type A has been described as the strongest toxin and a lethal dose for a person of 75 kilos has been estimated at approximately $0.15\ 10^{-6}$ gram.

Cl. botulinum of type E was demonstrated for the first time as late as in 1937 and the toxin has not yet been purely produced. High mortality in connection with E-botulism indicate that it has high toxicity. Laboratory cultures of type E have, however, so far given very low toxin results, when compared with cultures of types A, B, C, and D. It is possible that man is more sensitive to this toxin than the experimental animals usually used.

The botulinus toxin is quickly destroyed by heat. Type A toxin is destroyed at pH 5.0 when the heating reaches 80°C. In experiments by Bonventre and Kempe, their rod of Cl. botulinum, type B, grew in temperatures up to 48°C, but at that temperature the toxin was inactivated in part. At temperatures between 10 - 18°C a certain growth and toxin formation took place. The toxin was at these temperatures very stable, and, therefore, an extended incubation period had a lengthening effect on the toxin (Fig. 1)

In the experiments of these scientists the culture filtrate of Cl. botulinum contained after 190 hours toxin with a strength of 500 mes. Minimal Lethal Dose (M.L.D.).

Scott and Steward, in 1950, noted that preserved vegetables contained substances which protected the toxin from falling apart when heated. The heat stability varied for different products. The protective effect of various ions is highest in solutions with high ion strength. Especially Ca^{++} and Mg^{++} ions have proved to have great protective effect.

In the literature the botulinum toxin is traditionally described as toxic when orally consumed because it is not affected by the digestive juice.

Halliwell, in 1954, treated pure, crystallized type A toxin with pepsin, trypsin, and papain and found that papain had no inactivating effect, that pepsin caused weak detoxification, while the toxicity was much decreased by trypsin.

Pappenheimer, in 1948, pointed out that it is difficult to imagine that a substance with as high a molecular weight as the botulinum toxin could be absorbed from the intestinal region, and thinks it plausible that the toxic molecule is in part broken up into toxic fragments by proteolytic enzymes.

Coleman, in 1954, reported that crystallized A-toxin had no poisonous effect on mice, when given orally. The experimental animals were, on the other hand, killed by intraperitoneal injections of the same.

When evaluating these results we must take into consideration that earlier observations of the oral toxicity of the botulinum toxin on the whole are based on relatively unprepared toxin filtrates. Coleman hints at the possibility that some unknown factor which gives the toxin the oral effect is lost in isolating and purifying processes.

It is reported that ethyl alcohol can break up toxin in model experiments. People who consumed alcohol in connection with meals, after which cases of botulism occurred, have in many cases survived, while their more sober neighbors at table lost their lives. Uneven distribution of the toxin in the food may nautrally also have an effect, as well as differences in resistance to the toxin in different persons.

The information about the effect of NaCl upon the growth and toxin formation of Cl. botulium varies somewhat in the work of different authors. Tanner and Evans, in 1934, reported that 5 our of 6 rods that they had studied were completely stopped in their growth only when the NaCl concentrations reached 8.5 - 10.5%. They also found toxin formation without noticeable growth at 10.5% salt. Other scientists, Scott for instance, in 1955, found that 5% salt wholly stopped the growth while the toxin formation

stopped at 6% NaCl. The limit values of salt tolerance are to some extent dependent upon the nutrient medium in which the organism has been cultivated.

Cl. botulinum lives saprophytically and does not give rise to infections with consequent secondary cases. Botulism is thus a poisoning due to consuming of the botulinum toxin. The food in question does not need to include vegetative cells in order to be poisonous. Only brain and nerve tissues seem to be affected by botulism. It has been reported in some cases that Cl. botulinum can infect wounds dirtied by soil. Different animal species vary with regard to receptivity to the botulinum toxin. Guinea pigs are, for example, twice as sensitive as mice. Per unit of weight, man is as receptive as mice.

The formaldehyde toxoid can be used as an immunizing antigen to bring about active immunity from the botulinum toxin. This method has been tested on cattle and sheep in Australia.

The botulinum toxin is a good antigen and antitoxic sera with high titers can be produced. Under experimental conditions these antitoxins have prophylactic value.

Cl. botulinum in nature

Cl. botulinum has been isolated from soil in various parts of the world. Type B has been isolated in soil from Belgium, Denmark, England, and Holland, and type A was isolated in Swedish soil specimens by Faehraeus in 1949 and Dubowsky and Meyer in 1922, who found that types A and B were relatively universal, while C was only detected in certain special areas. Type E, which has most often been noted in connection with botulism in fish products, was isolated by Dolman in 1957 in a soil specimen from Canada, and Pedersen, in 1954, found Cl. botulinum, type E, in 23 out of 60 soil and slime specimens from the ocean bottom.

The ways of the spreading of Cl. botulinum in nature are in part unknown, but several observations seem to show that the organism is spread in insects, rats, birds, and fish. Information to this effect has been reported by Bengtsson, in 1922, who isolated Cl. botulinum, type C, from fly larvae (Lucilia caesar) and who, furthermore, noticed that chickens who ate such larvae often died, showing symptoms which resembled those which are described as typical of botulism.

Gunderson found in 1935 that a beetle of the Enochrus species contained toxin of type C. In 1925 it was reported that approximately one million birds were killed by botulism in a single epidemic in eastern Oregon of the US. In the US, a comprehensive study of the frequency of botulism in sea birds has been conducted for some years under the leadership of the Public Health Service and the Fish and Wildlife Service.

In South Africa and America botulism in cattle has long been a great problem. The disease has been concentrated in areas where there is a lack of phosphorus in the soil, and the animals are then often forced to turn to the bones of dead animals to get the phosphorus they need. These dead animals have often proved to be infected by Cl. botulinum, and toxin and spores can thus be transferred to healthy animals.

Rats are reported to have a high natural resistance to botulism and excrement of apparently healthy rats has proved to contain spores of Cl. botulinum.

Finally it may be mentioned that a number of water plants have proved to be satisfactory media for growth and toxin formation of Cl. botulinum, for example, Typha latifolia, Phragmites communis and Scirpus species. Rotten parts of plants in water where there is shortage of oxygen may therefore be another ecological niche for the growth and spore formation of Cl. botulinum under natural conditions.

Articles of food which cause botulism

What risks are there that Cl. botulinum will grow and form toxin in articles of food which are either preserved in the home or commercially hermetically sealed?

To start out with commercially hermetically sealed food articles, it can be quite categorically answered that the risks are astronomically small. The fact that human botulism due to consumption of commercially canned food has not been reported over the last 25 years shows how safe our commercially produced food articles are from the point of view of botulism.

Food articles, hermetically sealed in homes, have during the same period of time caused a considerable number of cases of botulism, but also that aspect of the problem is not of much current interest.

The risk of botulism is a little greater when we come to fresh and pasteurized food articles (heat, chemicals, radiation) which normally demand refrigeration, but we must stress that here also we are only discussing theoretical risks. There are no cases of botulism to verify our suspicions.

The greatest factor of uncertainty is perhaps that the methods which the industry has set up, on the basis of experience, to eliminate pathogenic organisms in food articles do not inform us of what safety factors in a certain process of a certain food article prevent the growth of Cl. botulinum. "Luncheon meat" -- to take a concrete example -- is normally sterile in spite of the fact that this product is not heat-treated at temperatures which alone could kill the bacterial spores. Is it then a cooperation between heat-treatment, added nitrate and nitrite, natural inhibitors in the substrate and pH that kills the spores, or is it a single one or a couple of these factors that are decisive?

Ball and Olsson sum up this problem in the following manner: "This is an example of taken for granted safety in foods, which our most advanced theoretical knowledge tries to convince us does not exist, but which experience, supplemented by actual scientific tests, shows does exist."

The background of this statement is, among other things, investigations by Ingram in 1957, which indicate that Clostridia are often present in raw meat and that Cl. botulinum survives long periods of time in brine to which nitrate and nitrite are added according to Beerens, 1957. Further aspects have been obtained from results by Greenberg and his collaborators, who noticed toxin formation in salt pork containing between 6.25 - 7.12% salt, without detecting any unpleasant smell. When the salt concentration, on the other hand, was below 5%, the toxin formation took place after the product had become unacceptable from an organoleptic point of view. According to these scientists, the salt concentration should therefore be above 9% or below 5%.

Some factors which decrease or increase the safety margin of botulism in food articles

Salt was in earlier times, on a much larger scale than now, a preservation means, which was used in concentrations sufficient to give the food article in question long microbiological stability even at room temperature. Today, salt is used in products, such as pork, corned beef, sausage, etc., more as a spice which gives the flavor that suits the consumer. Instead, heat pasteurization is used, which, however, is not sufficient to give satisfactory stability at room temperature. Refrigeration is a necessary part of the work to increase the stability of a food article. If these food articles are kept at too high a temperature for a long time, there is at least a theoretical risk of botulism. That the risk is not especially great is perhaps proved by the fact that only one case of botulism caused by commercially manufactured, pasteurized pork -- which industrially seen is a great product -- has been reported.

Factors which apparently also very effectively prevent growth and toxin formation in certain food articles are also their contents of growth inhibitors, or their lack of growth factors, necessary for the formation of Cl. botulinum.

Spores of Cl. botulinum and other aneerobes have been isolated in fresh milk, and cheese produced therefrom probably often contains botulinum spores. Nevertheless, only one case of botulism has been reported after consumption of commerically produced cheese. In an experiment in 1956, Wagenaar and Dack found that cheeses which had been inoculated with 10,000 spores/g of a blend of types A and B did not contain any toxin, even after preservation up to six months at 90° F (32°C). The number of living spores even decreased successively during the preservation period. It has been proposed that unsaturated fatty acids, produced during the ripening of the cheese, prevent outgrowth and growth of spores in cheeses.

As far as is known, there is no reported case of botulism in connection with commercially produced, so-called half-preserves, of fish. It is not clear what constitutes the safety factor in this case, but it is probable that it is the combination of salt, preservatives, and a relatively low pH that gives a satisfactory safety margin. The bacteriocide effect of benzoic acid, which is the most used preservative in this connection, is considerably greater in the presence of NaCl in the nutrient substrate, according to what we have experienced at the SIK (The Swedish Institute of Preservation Research). NaCl concentrations, which alone do not prevent the growth of test bacteria, also enhance very much the effect of benzoic acid.

In homes and to some extent in bigger households, fresh herring is still put in a marinade where still most often the only preservative used is vinegar. These marinades are often mostly anaeorobic. Even if the vinegar in these marinades decreases the pH to acid levels which makes outgrowth of Cl. botulinum impossible, the conditions within the swelling fish muscle may be favorable to outgrowth during a sufficiently long period to admit toxin formation. This can happen very quickly, which is demonstrated by a case in Germany where botulism developed 15 hours after consumption of pickled herring, marinated in vinegar for four days before the consumption.

Also the potential risk that botulinum toxin will develop in deep-frozen fresh foods, if these, before refrigeration or after defrosting, have been badly maltreated, has been discussed by a series of food bacteriologists. Ready-to-eat deep-frozen dishes which usually receive only a relatively mild heat treatment have also been paid attention to in this connection. The heating that takes place in this case is not sufficient to kill spores of Cl. botulinum possibly present, and since the saprophites which are antagonistic to Cl. botulinum are more or less completely killed off by the heating, there is the risk that toxin would develop in many such substrates if the dishes are kept for a long time after defrosting.

The examples I have mentioned earlier of the psychrophilic character of certain Cl. botulinum rods which makes outgrowth possible down to +10° C, and the observation by Ohye and Scott in 1953 that certain E rods can grow all the way down to +5° C show that this condition can be of a certain importance.

Thus, Tanner and collaborators found in 1940 that deep-frozen spinach and beef which had been inoculated with Cl. botulinum and then defrosted and kept at +10° C were toxic after four days. In these cases there was little break-up of the food article. Straka and James found in 1935 that out of 83 inoculated specimens of deep-frozen peas which had been kept for a week at +10°C, one was slightly toxic. Saleh and Ordal found in 1935 in their experiments that when defrosted specimens of deep-frozen chicken were kept at 30° C, toxin developed in three cases of 12 specimens which were not inoculated in advance. This suggests that infection of spores of Cl. botulinum in certain cases is not unusual.

The same scientists have, on the other hand, reported that no toxin was formed in chicken inoculated with Cl. botulinum spores, deep-frozen, and after defrosting, kept at +10° C.

An interesting possibility of increasing the safety margin with regard to botulism is sketched in a work by Saleh and Ordal. They account for tests when certain lactobacilli rods were put into deep-frozen chickens which were inoculated with Cl. botulinum. Even after five days' incubation at 30° C none of the 16 specimens which had been inoculated with both Strept. lactis and Cl. botulinum showed any toxicity. When Cl. botulinum alone was inoculated, toxin formation took place in many of the tests. The rod of Streptococcus lactis, which had a protective effect, produced antibiotic nisin, and therefore the protection may have originated not only from the decrease of pH due to produced lactic acid, but possibly also from the nisin.

The use of ionized radiation to increase the stability of certain foods is a new method, which will possibly be introduced on a commercial level. In this connection, among other things, the possibility of radiating deep-frozen foods to extend their stability after defrosting, which would be of great importance for the distribution of deep-frozen foods in many underdeveloped countries, has been discussed. The radiation doses would in these cases not be sufficient to kill the Cl. botulinum spores, but the vegetative cells of the saprophytic flora would partly be eliminated. Whether the extended stability acquired in this manner possibly influences the safety margin with regard to toxin formation by Cl. botulinum after defrosting must be investigated before such treatment is begun.

Human botulism is fortunately very rare and the disease is completely unknown in certain countries. The geographical distribution is very irregular. Thus, the reported cases of botulism in the US during the last 50 years are to a very high degree concentrated on the West Coast, while only occasional instances are reported from the central and eastern areas.

Most cases in the US have been caused by home-preserved vegetables; string beans, corn, and spinach have especially been pointed out. These food articles seem often to offer a suitable milieu for toxin formation before the present saprophytic flora has made them inedible from the point of view of taste. Corruption of meat products on the whole takes place faster, and therefore the risk of running across a meat product which is acceptable from the point of view of taste and contains botulinus toxin is possibly less great.

The frequency of botulism in the US during the years 1889-1949 is shown by Mayer in 1950; see Fig. 2.

The frequency of botulism, divided among various foods, appears from Chart 1. As can be seen, spring beans, corn, and spinach are responsible for most cases.

The commercially produced food articles which in certain contexts gave rise to botulism in the 1920s were in the first place spinach and olives.

Imported champignon mushrooms, canned in the US, and imported, canned sprats from Germany were responsible for other cases. Most cases in the US were caused by toxin type A.

Most cases of reported cases of botulism in Europe were in connection with consumption of meat products, and most often it has been toxin of type B that has been detected. On the whole, mortality has been considerably higher in the US than in Europe.

Further, data regarding cases of human botulism from all over the world are put together in Chart 2, according to Meyer, 1956.

Of special interest is the great number of cases reported from France in the years of 1940-1944. Most cases were, however, not serious and only about 2% of these were fatal. In Charts 3 and 4 we find the frequency of botulism in Norway and Denmark. These data we got from Dr. Skulberg with regard to Norway and from Dr. Bang Jensen with regard to Denmark. The Scandinavian cases were to a great extent caused by fish products.

Many cases of botulism are also reported from Russia. Thus, the first culture of type E was isolated from sturgeon in the Ukraine. In 1927 it was reported that botulism spread more and more in the USSR and that there had been difficult outbreaks in, among other places, Odessa and Charkow. Unfortunately, there is no statistical information about the development in that part of the world after 1927.

Botulism, caused by toxin of type E, has since 1927, when this type was first isolated, been reported from the US, Canada, and Japan, and in all cases after consumption of fish products.

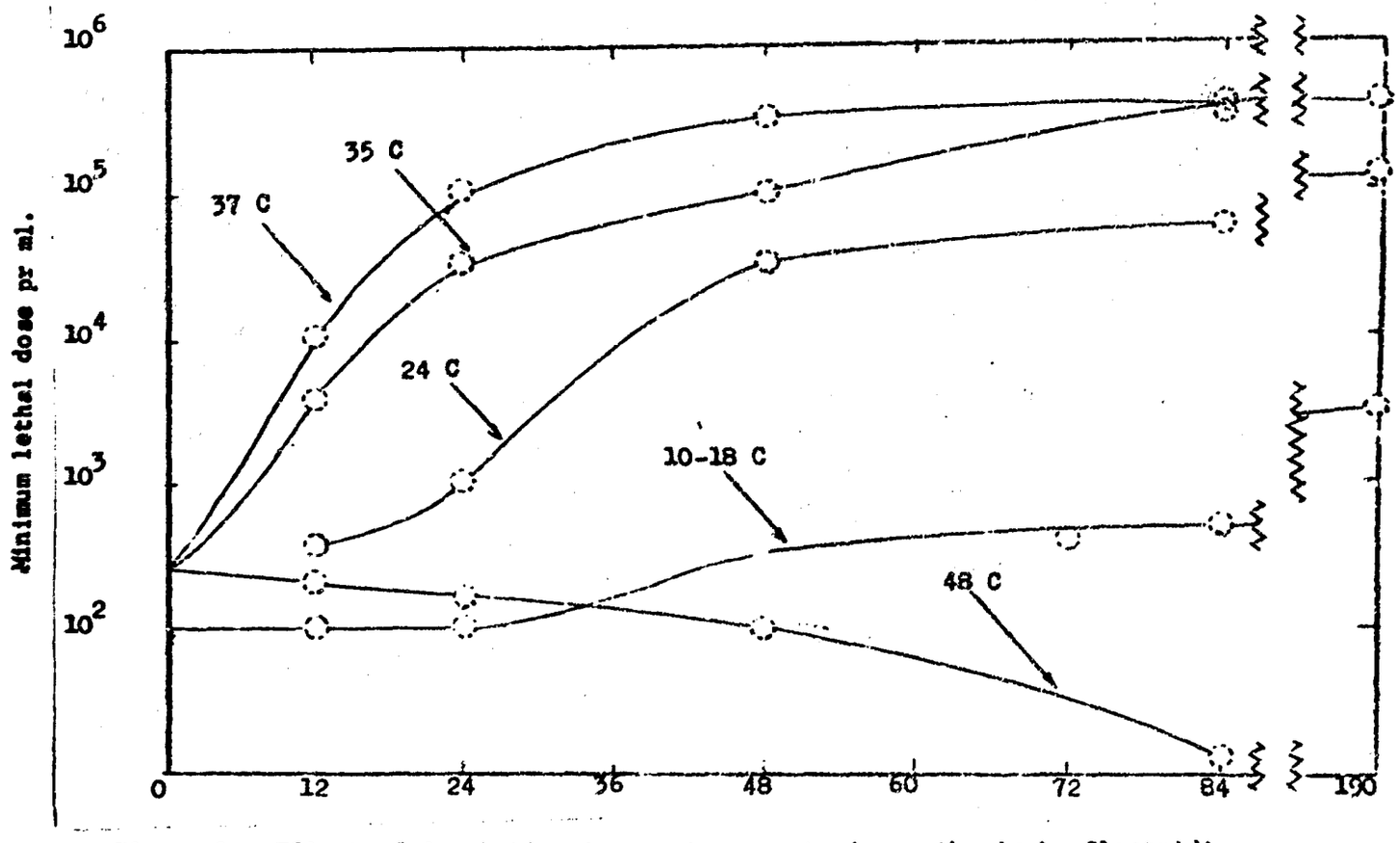


Figure 1. Effect of incubation temperature on toxin synthesis by Clostridium strain JTD IV from Bonventre and Kempe, 1960.

Figure 2. FIFTY YEARS OF BOTULISM IN THE UNITED STATES AND CANADA

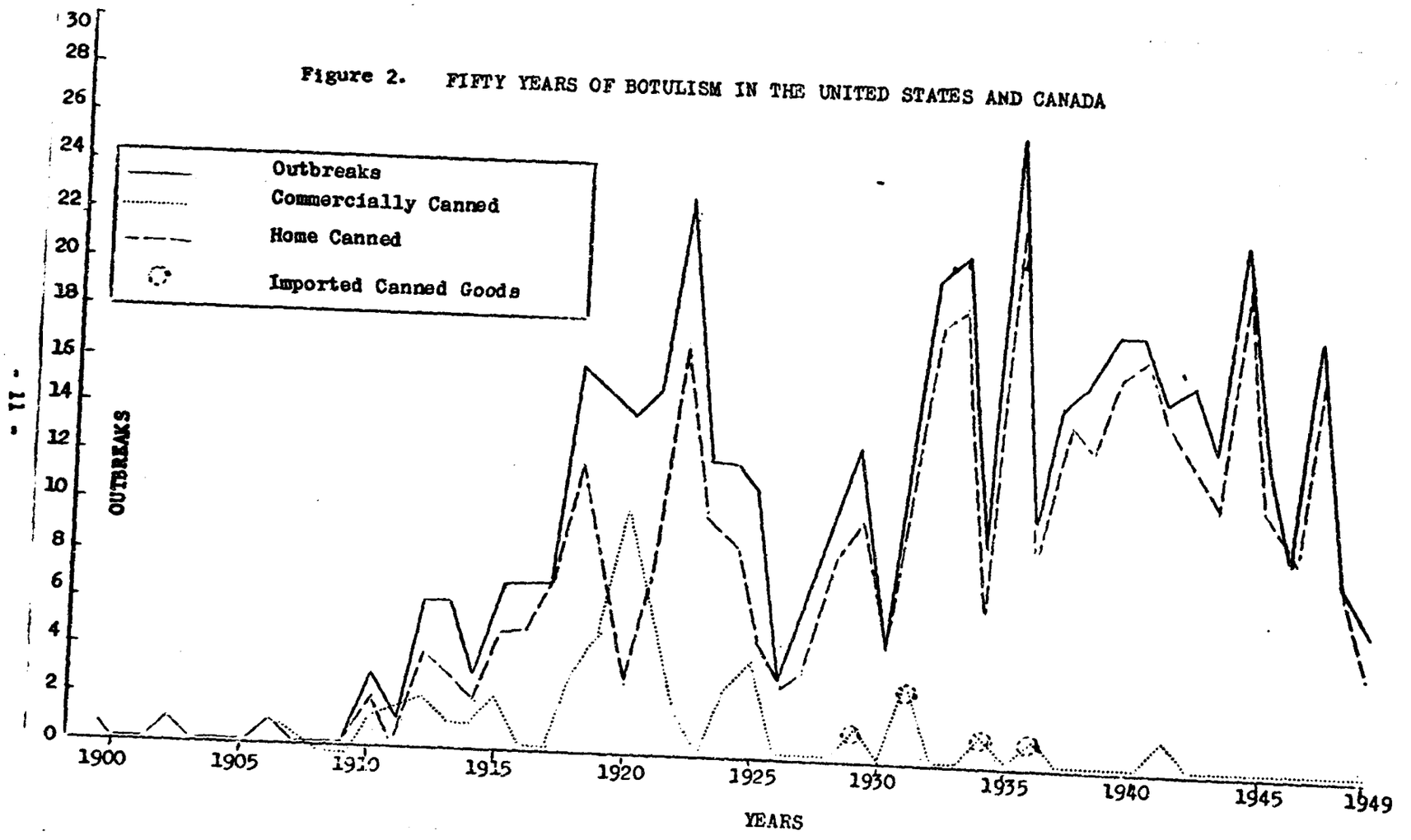


TABLE 1

FOODS INVOLVED IN OUTBREAKS OF BOTULISM IN US AND CANADA
1899 - 1949 (Meyer, 1950)

Foods	Out-breaks	Cases	Deaths	
Vegetables				
String beans	98	246	189	Three comm. packed
Corn	49	100	72	One " "
Spinach or chard	24	102	50	Ten " "
Beets	24	91	54	Two " "
Asparagus	23	53	42	Home canned
Olives	14	73	40	Twelve comm.packed
Chili peppers	14	55	37	Home canned
Peas	12	37	17	" "
Figs	11	22	12	" "
Beans	9	18	11	" "
Beet tops	9	28	18	" "
Tomatoes	8	23	12	" "
Mushrooms	7	30	10	" "
Apricots	4	18	13	" "
Okra	3	7	2	" "
Pears	3	3	2	" "
Meats				
Sausage	6	9	5	Two comm. preserved
Ham	5	21	18	One " "
Beef	5	16	9	Home preserved
Chicken	3	3	0	" "
Pork products	3	10	7	One comm. packed
Blood sausage	2	5	5	Home preserved
Lamb stew	1	1	0	Home canned

(continued)

TABLE 1 (continued)

Foods	Out-breaks	Cases	Deaths	
Sea foods				
Salmon	8	21	9	One comm. canned
Tuna	7	24	14	Three " "
Clams	5	12	10	Four " "
Sardines	4	7	6	Three " "
Milk products				
Cheese	5	18	8	Home prepared
Milk	2	5	0	Two comm. canned

TABLE 2

GEOGRAPHICAL DISTRIBUTION, INCIDENCE, AND NATURE OF BOTULINOGENIC FOOD

Country	Period	Source of figures	Number of epi-sodes	Number of cases	Number of deaths	Type of botuliogenic food (confirmed)	
						animal products	vegetable products
Europe							
Austria-Hungary	1891-1909	Compiled	4	14(?)		4	
Belgium	1894-1906	"	3	38	3	3	
Chechoslovakia	1921	Sieber	1	2	0		
Denmark	1906-46	Dyggve	4	13	3	4	
"	1951	Pedersen	1	6	0	1 (fish)	
France	1875-1936	Compiled		24	3		
"	1940-44	Légroux et al.	500 (approx.)	1000	15	163 (93 % pork products)	6
"	1945-48	Verge	85				
"	1950-54	Compiled	5	26	2	5 (5)	
Germany	1898-1923	Knorr	77	344	60	74	3
"	1929-31	Meyer	33			33	
"	1933-38	"	153	414	41	153 (14)	1
"	1943-47	"	18	53	10	18 (58)	
"	1948	"	10	33	2	7 (3 fish)	
"	1949	Farchmin	9	13(?)	13	6 (fish)	
"	1923-48	Verge	357	950	119	307	

(continued)

TABLE 2 (continued)

Country	Period	Source of figures	Number of epi-sodes	Number of Cases	Number of Deaths	Type of botuliogenic food (confirmed)	
						animal products	vegetable products
Great Britain	1922-54	Compiled	7	15	13	7	
Italy	1903-22	Pitini Pisani	2	5	1	2	(1)
Netherlands	1935-54	Compiled	5	12	2	5	
Norway	1934-42	"	4	10	3 (?)	3	
Poland	1920-26	"		20	14		
Sweden	1933-49	"	4	11			
Switzerland	1885-1932	"	3	36	5		
Russia	1878-91	"		288	111		
"	1818-1913	Matviev		609	283	fish poi-soning	
USSR	1917-26			52	35		
"	1937	Zavadovskaya (quoted by Dolman & Chang)		168	56		
Yugoslavia	1916	Novotny & Ringel	1	1			
South America							
Argentina	1920-26	Pando	2	8+	7+	3	
North America							
Canada	1919-54	Dolman	14	63	35	3(meat) 5(fish)	6
USA	1899-1954	Meyer & Eddie	514	1350	861	38 (meat) 28 (fish) 8 (milk)	38?
Australia	1948	Gray	2	32	7		2
Japan	1954	Matsui	6	25	10	6 (fish)	

Bull. Wld Hlth Org. 15 (1956) 281-298

A. SKULBERG "HUMAN BOTULISM IN NORWAY." SHORT SUMMARY OF OUTBREAKS

(To be published in Nordisk Veterinärmedicin 1960 - 1961)

Outbreak No.	Year	Number of cases	Incriminated food	Diagnosis
1	1934	5	Salted and dried ham	Botulinum toxin demonstrated, but no type identification.
2	1942	1	Home canned hamburgers	Diagnosis based on clinical symtoms.
3	1943	2	Home canned fish cakes	-"-
4	1947	1	Home canned hamburgers	-"-
5	1947	5	Salted and dried beef	Botulinum toxin type B demonstrated
6	1954	33	Salted and dried ham	-"-
7	1955	1	Home canned hamburgers	-"-
8	1956	3	Salted and dried ham	-"-
9	1957	1	"Rakefisk" x)	-"-
10	1959	1	"Rakefisk" x)	-"-

x) Fermented fish (Salmo trutta) eaten without preceding heat treatment.

TABLE 4

REVIEW OF CASES OF BOTULISM IN DENMARK

Year	Reported By	Persons Made Ill	Fatalities	Food
1901	l'h. Madsen	4	4	Salted mackerel
1913	N. Höeg	1	0	Canned salmon
1930	C. Schondel	2	2	Boiled liver paste
1938	M. Trane	6	0	?
1938	C. Schondel	2	0	?
1939	C. Schondel	3	3	Salted herring
1946	H. Dryggve	2	2	Salted herring
1951	J. Pedersen & A. Christensen	6	0	Fresh herring in oil
1956	Bang Jensen & F. Hahnemann	2	1	?
1958	Bang Jensen & F. Hahnemann	4	1	Boiled liver paste
1901-1958		32	13	

Börge Bang Jensen og Fin Hahnemann (1959) Ugeskr. Laeger 121/36

A PAPER ON INFECTION AND TOXIN FORMATION BY CLOSTRIDIUM BOTULINUM, TYPE E, IN FOOD ARTICLES AND AN EVALUATION OF PREVENTIVE MEASURES AGAINST IT

by

H. O. Pedersen

If I were asked to classify the botulinum bacteria, I would divide them into two main groups, of which one would comprise types A, B, C, and D and be designated the terrestrial botulinus group and the other type E with the designation of the marine botulinus group. Additional designations would refer to the natural environment of the bacteria -- in the first place land and sea.

I shall try to justify my audacious statement by giving some essential facts.

Since I shall in this paper exclusively deal with type E, I want to mention that in 1936 it was classified as a special type by Gunnison, Cummings, and Meyer (1937).

As we know, the natural environment of the botulinus bacteria has up to now been considered to be terrestrial. However, this does not seem to bear upon type E. Thus, both Danish (1954) and Japanese (1956) investigations have shown that type E appears in unusual density in mud and sand in coast areas and even in deep-water silts.

The chart shows the frequent appearance of type E within the harbor area of Copenhagen (Chart 1).

That water-dwelling creatures are apt to get type E mixed with their intestinal flora is then not so remarkable, and there are also several scientists, Dolman (1957), Prevot (1951) Nakamura and others (1956) who have isolated this bacterium from the intestinal contents of fish.

These investigations constitute the essential basis when we count type E as a special marine botulinus group.

It is probable that the E spores are very unevenly distributed in the various ocean areas. At present it is believed that the spores appear with great density in ocean areas which border Canada, Greenland, Japan, and Denmark. On the other hand, investigations of coast areas in Australia have given negative results with regard to E-spores (1957).

One can naturally not sharply differentiate between terrestrial and marine areas, since soil particles to a great extent are brought to the oceans through floods and watercourses, just like, on the other hand, infected materials are brought in many ways from the oceans to land areas. Type E has thus also repeatedly been pointed out in investigations of soil specimens (Dolman 1947, Pedersen 1954).

If the above observations are correct, the natural conclusion should be that it is exclusively, or almost exclusively, marine products that become infected with type E and cause E-botulism in people, and that is the true situation.

Dolman (1953, 1957) has, at some years' interval, mapped all known cases of E-botulism. Until 1953 there were only 10 outbreaks of E-botulism reported. At the present time the number of outbreaks has increased to about 40 and there will surely steadily be new cases.

If we investigate what food articles have caused the intoxication we find that it has been in all instances marine products. In most cases it has been fish or roe which has been treated or prepared in some way, but not heated. Thus, it has been raw products. Defective, home-cooked fish and raw meat of ocean mammiferous animals like the white whale have also in some cases been involved in the outbreaks.

The ways of treatment of the food articles have on many occasions been peculiar and hap-hazard, but what is common to them all is that they have never been eaten immediately after the preparation and that they have even been left to ferment for a long time.

Most of the cases of E-botulism have occurred among peoples (Indians, Eskimos, and Japanese) where the understanding of basic hygienic concepts is slight, but there have also been cases in such enlightened countries as the Scandinavian.

The special circumstances which play a role here are that a number of people -- perhaps most people -- like to eat raw fish in one way or another.

It may be surmised that in many cases the fish while alive is infected through infected water or infected food, but, on the other hand, it is also possible that the bacteria are acquired in connection with the cleansing and processing, among other things, through hands and tools which have been in contact with infected material. It cannot be excluded either that the formation of toxin can take place in fish refuse and rooms which have not been sufficiently cleaned.

Even if type E has many characteristics in common with the other types, one will find when investigating its physiology that on several essential points it possesses its own distinctive stamp.

An especially important genetic factor is the temperature zone of growth of the bacteria. Ohye and Scott (1957) have investigated the growth of ten type E rods at temperatures between 2.5 and 45° C.

The lowest temperature for formation of spores and growth was 5° C and the highest 45° C, but at that high temperature only 5 of the 10 investigated rods showed signs of growth. The biggest cell yield and the strongest growth is reached at 35° C where there is about 1.8 divisions per hour. At 37.5° C the temperature coefficient is already negative and the growth curve goes steadily downwards. Ohye and Scott (1953) have also investigated the growth of twenty A and B rods and established that these two types give almost identical results (Fig. 1).

If one compares the growth curve of E with that of A + B, one will notice that the minimum temperature for growth of E rods is 8-10° C lower than that of A + B. The other growth temperatures likewise show shifts (5-7° C) toward a lower temperature zone.

Another important difference between type E and the other types is the temperature zone of toxin formation.

It is found out that if one grows type E at 37° or at higher temperatures over a longer period of time, one will have no or only a little toxin yield. I have investigated this matter more closely with regard to 3 rods.

As can be seen from the curves (Fig. 2) there is a moderate toxin production during the first days, but in the course of about 8 days there is literally only a backward trend.

Something similar is apparent in the table (Table 1) -- cultures grown at 37° C do not yield any toxin.

If one, on the other hand, lowers the growing temperature to 30° C or below, there does not seem to be any apparent disturbance of the toxin production

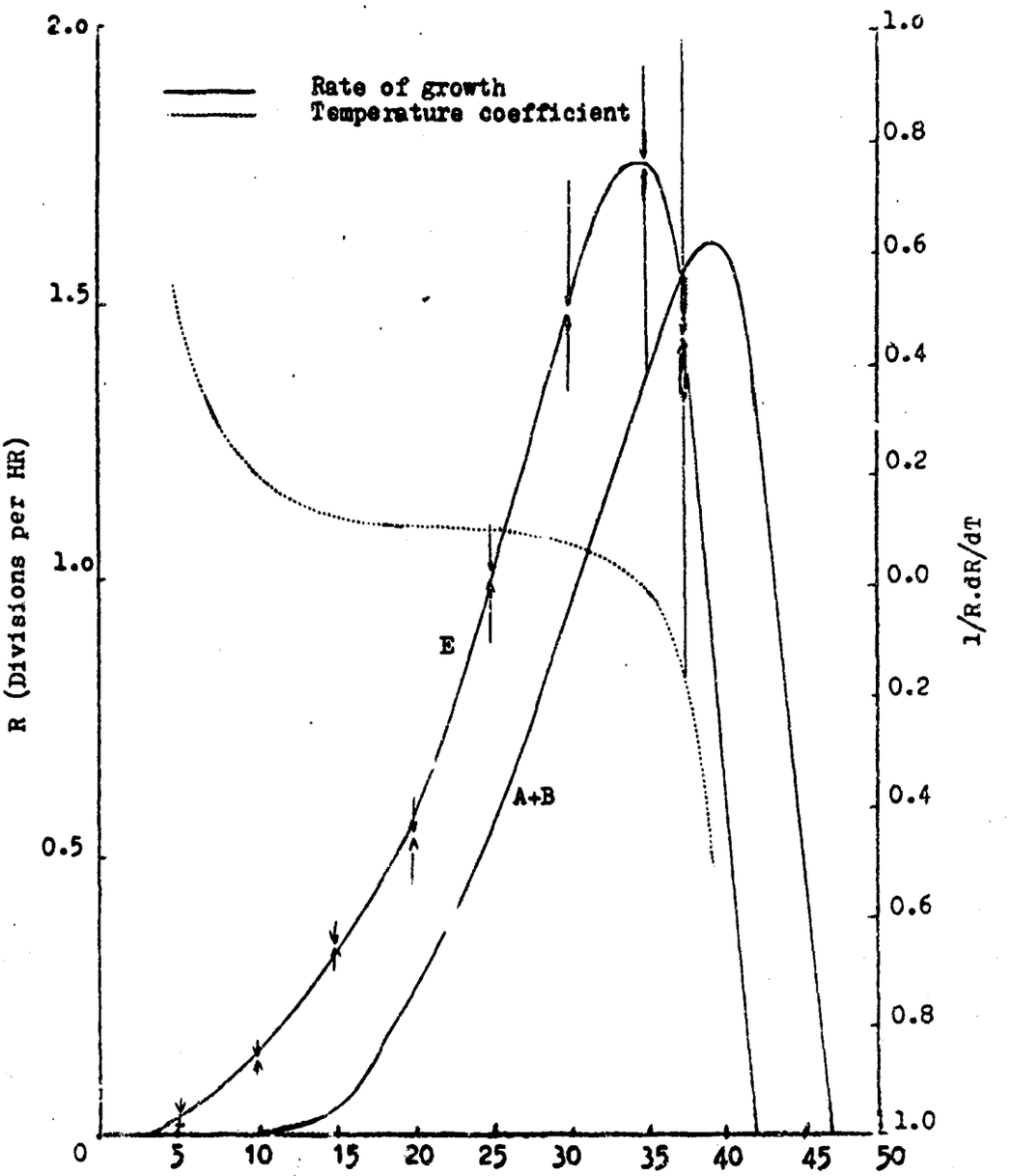


Figure 1 - Relation between the mean rate of growth (R), the temperature coefficient of R, and the temperature (t) in N.Y.G. medium. The heavy vertical lines show the mean value of R $\pm$ its standard error; the light vertical lines show the range of values observed at each temperature. The curve A and B shows the mean values of R previously obtained for types A and B strains (Ohye and Scott 1953).

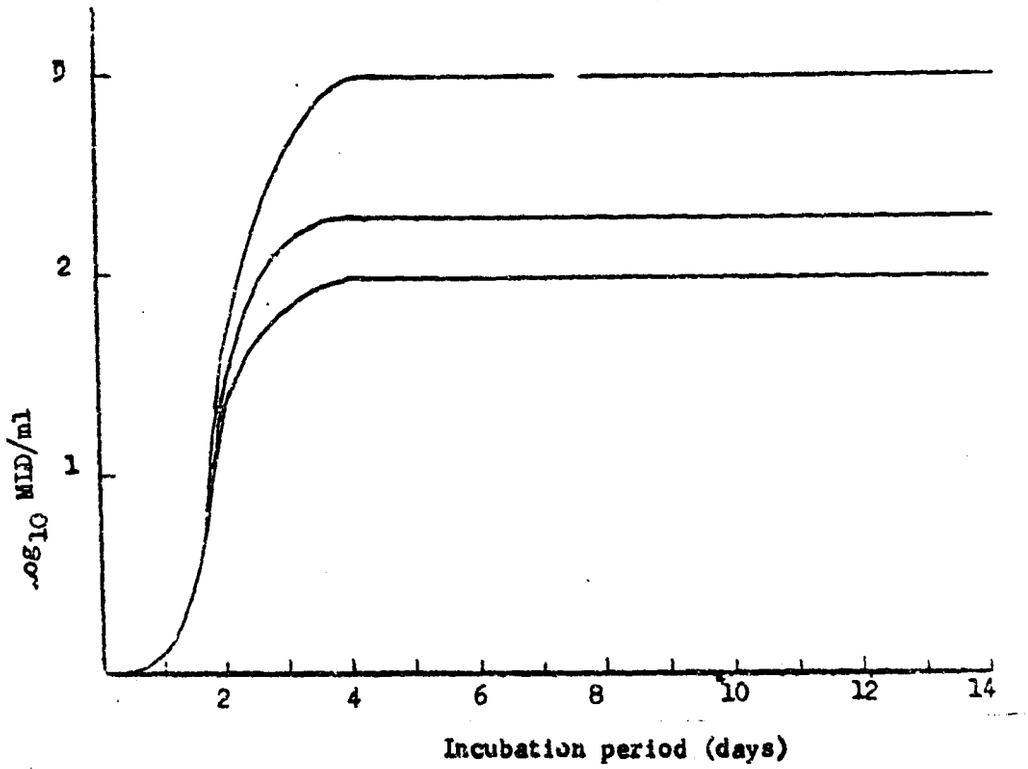


Figure 2 - The toxigenic activities of three type E strains at 30°

This figure (Fig. 3) shows toxin production at 30° C. The maximal toxin amount is reached after about 4 days and it keeps constant over a longer period of time. The curves show further that the individual rods display differences depending on the substances in which the toxin formation takes place.

Since there may be a connection between the growth of the bacteria and the toxin production, one can surmise that the unstable toxin production at 37° C is an expression of the great disturbances which affect the bacteria's growth at that temperature. This is also in harmony with the fact that the temperature coefficient has become negative.

It is generally recognized that the botulinus types A and B produce very thermoresistent spores - spores which can endure cooking for a long time. Type E spores are not at all in possession of this quality. On the contrary, when it comes to heating, the E spores belong among the very sensitive bacterial spores.

TABLE 1

INVESTIGATIONS OF E-TOXIN IN PARALLEL CULTURES DROM DREDGE MUD, PARTLY HEATED TO 80° C FOR FIVE MINUTES AND CULTIVATED AT 23° C and 37° C

Place of Origin of Specimen	No. of Specimen	Cultivation Temperature	Cultivation Days	Material	E-toxin D.M.L. pr. ml culture	Cultivation Days	Material	E-toxin D.M.L. pr. ml culture	Cultivation Temperature	Cultivation Days	Material	E-toxin D.M.L. pr. ml culture	Cultivation Days	Material	E-toxin D.M.L. pr. ml culture
outside															
Assistenshuset	41	23°C	8		20	8		0	37°C	8		0	8		0
Gl. Strand 52	42	23°C	8		20	8		0	37°C	8		0	8		0
Gl. Strand 46	43	23°C	8		20	8		100	37°C	8		0	8		0
Gl. Strand 42	44	23°C	8		20	8		0	37°C	8		0	8		0
Gl. Strand 36	45	23°C	8		5	8		0	37°C	8		0	8		0
Gl. Strand 28	46	23°C	8		100	8		0	37°C	8		0	8		0
Absaion	47	23°C	8		20	8		0	37°C	8		0	8		0
Nybrogade	48	23°C	8		100	8		0	37°C	8		0	8		0
Knabrostraede	49	23°C	8		20	8		0	37°C	8		0	8		0
Nybrogade 16	50	23°C	8		200	8		5	37°C	8		0	8		0
Lynettehavnen	51	23°C	9		20	9		0	37°C	50		0	50		0
Lynettehavnen	52	23°C	9		100	9		0	37°C	50		0	50		0
Lynettehavnen	53	23°C	9		200	9		0	37°C	50		0	50		0

Not Heated | Heated | Not Heated | Heated

The statements made by various scientists with regard to the destruction of E-spores by heat vary somewhat, which is not remarkable when one takes into consideration that most investigations have been based on cultures in which the number of spores has not been known. There is, however, so much agreement between the various investigations that one can say it would hardly be possible to find liveable E-spores after the materials have been heated at 80° C for 30 minutes.

I shall refer briefly to some investigations in this field made by Scott (and collaborators) (1957).

Unfortunately, the investigation only concerns two rods. They found that the logarithm of the number of living spores at 3 different destruction temperatures was a linear function of the time. The decimation of the number of spores at 80° C with regard to the two rods took place in the course of respectively 3.3 and 0.4 minutes. The spores of one rod were thus somewhat more resistent than the other's. The slight heat resistance of the spores was shown by the fact that the time of decimation of the most resistent E-rods had to be given as 2 seconds at 100° C, which corresponded to 1/1000 of the spore existence of some A and B rods investigated.

What one is able to conjure out of bacterial spores in the laboratory, is one thing, however, how things occur in nature is another.

In my investigations of ocean dredge mud specimens I noticed that out of 19 specimens, which unheated, all produced toxin, there were only two that could do this after being heated at 80° C for five minutes during the cultivation (Table 1).

This would mean that only 4 or 5% of the spores in a natural environment have been able to survive for five minutes at 80° C.

I had thought that the pronounced sensitivity of the spores to heat must be expression of the fact that the E-spores in other respects were also slightly resistent. Thus, I considered it possible that the strong brine frequently used by the industry for salting of fish and meat might destroy the spores. Tests in this respect, however, gave negative results. I used six different brines containing between 15-22.4% NaCl; five of the brines contained nitrite in concentrations between 0.017 - 0.084% (1957). Each brine was inoculated with a relatively small amount of botulinous spores. The temperature during the test was 10° C.

As can be seen from Table 2, the number of viable spores after one month was really the same as at the beginning of the test; the shifts in the number of spores during the test were wholly blamed on technical irregularities.

In contrast, the A and B spores can germinate at respectively 7.3 and 6.3% salt. but the spores have, however, a rather long dormant period.

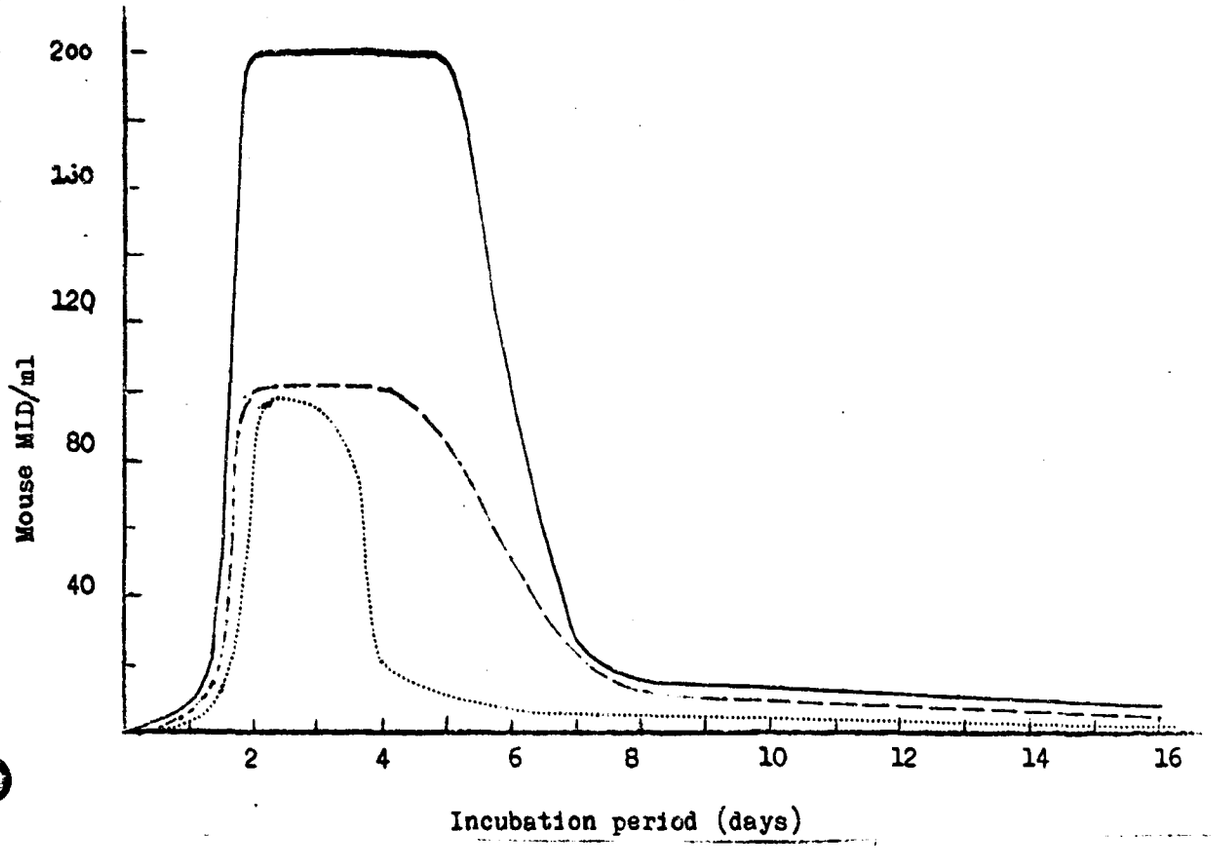


Figure 3 - The toxigenic activities of three type E strains at 37°. Continuous line, "Greenland" strain; broken line, "Gl. Strand" strain; dotted line, "Frederiksberg" strain.

The investigations have concerned the spores exclusively, because it is most probably that botulinus infection of food articles can only be caused by means of the spores since the vegetative cells are very sensitive to oxygen and therefore usually disappear before growth conditions are attained.

Finally I would like to make the following general remarks:

1. Cl. botulinum, type E, is a marine bacterium, and infection thereby seems therefore almost exclusively to involve marine products -- one should however remember that meat can possibly be infected by spore-carrying dust from occasionally infected land areas or due to the fact that the food article contains infected fish (for instance, braunschweiger).

2. Cl. botulinum, type E, can grow and produce toxin at temperatures as low as 5-6° C, but the amounts produced at temperatures this low will probably be small enough not to do any damage. On the other hand, I consider temperatures around 10-15° C sufficient to enable the production of lethal doses of toxin.

3. It takes about 4 days at 30° C in the laboratory to attain maximal toxin production, but one mortal outbreak of E-boutlism caused by infected herring which had been kept for 3-4 days at about 20-22° C was reported.

4. The E-spores are enormously thermolabile, and a heating of the food articles at 80° C for 30 minutes will almost certainly destroy the spores.

5. In case my investigations of the sensitivity of the E-spores to salt (NaCl) can be verified by other laboratories in experiments which deal with greater numbers of bacterial rods, a salt concentration of about 4% in the product will prevent germination of spores.

6. The boutlinus toxin is sensitive to heat and will be destroyed in the course of a few minutes at 80° C.

TABLE 2. The Survival of Cl. Botulinum in Brines

Type	No. of brine	Most probable numbers of spores/ml brine using 5 tubes with 0.1, 0.01 and 0.001 ml volumes. Testing intervals(days)				
		0-1	5-10	11-17	21-24	30-31
A	1	350	240	240	290	540
	2	540	130	170	240	540
	3	350	170	240	240	350
	4	350	1600	540	350	1600
	5	540	920	920	130	540
	6	1600	350	540	350	540
B	1	350	41	540	540	920
	2	240	180	540	540	540
	3	130	350	110	110	130
	4	350	240	220	79	170
	5	280	350	540	540	220
	6	240	350	920	350	240
E_1	1	170	79	350	540	240
	2	33	27	27	240	33
	3	350	170	540	240	130
	4	540	540	540	540	350
	5	130	49	220	130	79
	6	350	170	130	540	130
E_3	1	920	240	350	350	540
	2	920	350	540	540	240
	3	540	540	1600	350	350
	4	540	1600	1600	350	540
	5	1600	350	350	920	350
	6	350	170	540	350	1600

Comments:

On the other hand, it turned out in other experiments that the outgrowth of the E spores was very repressed by relatively low salt concentrations in contrast to the A and B types. Each culture was inoculated with about 500 spores.

From Table 3, it appears that about 4% salt totally represses germination and that at 3.9% there is pronounced dormancy.

TABLE 3

The Inhibitory Effect of Sodium Chloride Concentration on the Germination of Different Types of Cl. Botulinum Spores in Peptic Digest Liver Broth

Type	Salt (g/100 ml medium)		Time to visible growth (days)	Temp. (°C)
	Growth	No growth x)		
A	7·3	7·4	16	37
B	6·3	6·4	26	37
E_1	3·9	4·1	17	30
E_3	3·9	4·1	17	30

x) The negative cultures were observed during 30 days.

Literature

Dolman, C. E. 1957. Type E (fish-borne) botulism: A review. Jap. J. M. Sc. & Biol., 10, 383.

Dolman, C. E. & Kerr, D. E. 1947. Botulism in Canada, with report of a type E outbreak at Nanaimo, B. C. Canad. J. Publ. Hlth 38, 48.

Dolman, C. E. & Chang, H. 1953. The epidemiology and pathogenesis of type E and fishborne botulism. Canad. J. Publ. Hlth 44, 231.

Gunnison, J. B., Cummings, J. R. & Meyer, K. F. 1937. Clostridium botulinum type E. Proc. Soc. exp. Biol., N. Y., 35, 278.

Nakamura, Y., Iida, H., Saeki, K., Kansawa, K. and Karashimada, T. 1956. Type E botulism in Hokkaido, Japan. Jap. J. M. Sc. & Biol., 9, 45.

Ohye, D. F. & Scott, W. J. 1957. Studies in the physiology of clostridium botulinum type E. Australian J. Biol. Sc. 10, 85.

Ohye, D. F. & Scott, W. J. 1953. The temperature relations of clostridium botulinum, types A and B. Australian J. Biol. Sc. 6, 178.

Pedersen, H. O. 1954. Clostridium botulinum, type E: Investigation of the appearance in nature of the bacterium, and demonstration that too high a culture temperature (37° C) destroys the toxin production of the Danish E strain. VII, Nordic Veterinary Meeting, 472.

Pedersen, H. O. 1957. The survival of clostridium botulinum in curing brines. The microb. of Fish and Meat Curing Brines. Her Majesty's stationery Office, London, 289.

Prévot, A. R. & Huet, M. 1951. Existence en France du botulisme humain d'origine pisciaire et de Cl. botulinum E. Bull. Acad. nat. Med. 135, 432.

GAMMA RADIATION OF BOTULINUM TOXIN, TYPE A AND B

by

Anton Skulberg

Extensive research today is directed at preservation of food articles by means of ionized radiation. This research is performed in many countries and the objectives of the individual investigations are various. The main part of the research aims at clarifying the effect of ionized rays on micro-organisms. In this manner, sterilizing doses for the various bacteria can be ascertained or the ray doses that will destroy certain micro-organisms in a food and for example have a pasteurizing effect can be established. Other tests have as objective the mapping and clarification of the organoleptic changes that take place in radiated products. Comprehensive investigations, especially in the US, aim at finding out whether radiated foods may have damaging effects on the animal organism.

At Norway's Veterinary University we have undertaken investigations on the effect of ionized radiation on bacterial enzymes and toxins. I shall here deal more closely with some of the investigations which have concerned the botulinum toxin type A and B. The primary objective of these tests has been to examine the inactivation of botulinum toxin through gamma radiation. Such investigations seem to have their justification in the fact that ionized radiation may possibly be used in the preservation of food articles.

After initial investigations it was soon realized that we did not have adequate radiation resources in Norway for this purpose. Cooperation was therefore begun with the Low Temperature Research Station at the University of Cambridge so that the radiation could be undertaken in England. The material was sent by air in order that the investigation could be started about 24 hours after the radiation had been made.

For toxin production we used the rods "Hall" and "Beans" of respectively type A and B. We used Sterne and Wentzel's method (1950) according to which the toxin is produced in a "Cellophane bag" in a corn steep liquor medium. By choosing particularly toxogenic cultures of these bacterial rods we could attain toxin titers of the magnitude of 2×10^7 LD_{50} pr. ml for both types. Being fully developed, the cultures were centrifugalized and the super-natants were dialyzed in running water for 20 hours. To prevent inactivation glycerol was added to the dialysates in the proportion of 1:2 and stored at -20° C (Cardalla et al. 1958).

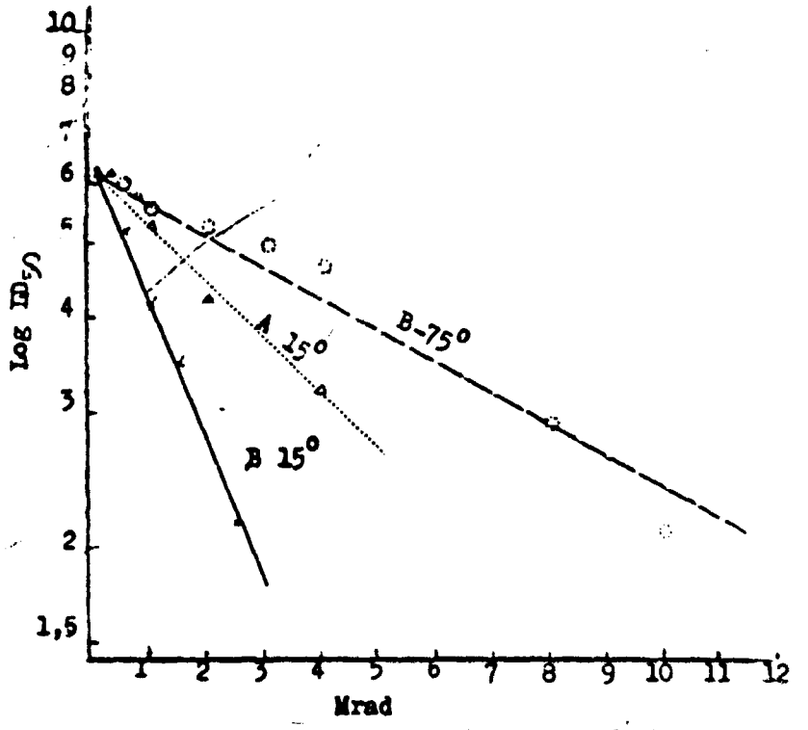
The radiation was undertaken at varying pH, and the toxins were therefore thinned by different buffers. A phosphate buffer with pH 6.2 and an acetate buffer with pH 4.8 and 3.8 were used. To stabilize the toxin, 0.2% gelatine was added to all buffers (Duff & al 1957 a and b). These buffers were also used as dilution liquids when the toxic activity was determined. The toxic activity in the solution of radiation was usually $2\ 10^6$ LD_{50} pr. ml.

After the radiation, the MLD situation was determined. The titration of the toxic activity was then undertaken at a LD_{50} determination. Eight mus (18 - 20 g) was used for each dilution group and 25% spacing between the groups. The LD_{50} values were established according to the method given by Reed and Munch (1938).

The toxin solutions were radiated in standardized glass-tubes with glass-walls of one mm. thickness. The radiation was undertaken from the side with 4 MeV electrons from a linear accelerator with a "dose rate" of 1 Mrad per minute. The radiation was undertaken under thermo-control. The investigations were made at two temperatures: +15° and -75°. At 15° the solutions were colled by means of running cold water and at -75° they were packed in powdered carbon dioxide. At 15° they were given doses of up to 4 Mrad and at -75° doses up to 12 Mrad.

Dosimetry was made by radiating solutions of cerium sulphate in 0.8 N sulphuric acid at 15° C in the same kind of glass-tubes at the same time as the toxin solutions (Tainuty & al 1959). See table and diagram.

Toxin type	Temp.	pH	D-values (Mrad)	Range of inactivation studied	Molecular weight (million)
A	15°	3.8 4.8	1.25	10^{-2}	1.6
A	15°	6.2	2.9	$10^{-1.5}$	0.65
B	15°	6.2	0.65	10^{-3}	1.3
B	-75°	6.2	4.12	$10^{-2.5}$	0.5



As can be seen from the diagram, there is an exponential relationship between the amount of toxin that is inactivated at the time of radiation and the absorbed ray dose. We have not been able to point out any significant deviation from this situation even in rather wide inactivating areas. The results can thus be explained on the basis of a "target theory," and we can assume that the radicals which are formed in the water probably react substantially on other components of the solutions without interfering with the toxin.

The table sums up the results. The D-values given in Mrad indicate the dose which inactivates 90% of the toxin, and the molecular weight is determined in accordance with the diagrams in the book "Actions of radiations on living cells" by D. E. Lea (Cambridge University Press, 2nd edition 1955). The results which are given in the table with regard to the molecular weight of type A toxin at pH 6.2 may be taken with some reservation, since there are indications that a fragmentation of the toxin molecule took place during the process. The investigations show, however, that a little toxin unit is not easily inactivated, which can be of a certain interest if one plans to use ionized radiation in the preservation of foods. It is doubtful whether the data, found at radiation of type B toxin at -75°, can serve as basis for calculation of the molecular weight because other factors also enter into the picture at this low temperature.

Earlier investigations were made by Wagenaar & Dack (1956). They measured the inactivation of type A toxin in cheese and in trypticase - beef heart medium. We cannot indicate satisfactory inactivation curves on the basis of these investigations, but they suggest that an exponential relationship between the absorbed radiation dose and the inactivation exists. In these tests, the D-values varied between 1.47 and 6.15 Mrad, and this gives molecular weights between 150 000 and 3 000 000, with an average of about 1 600 000.

The objectives of our investigations have been: First, to investigate the inactivation of botulinum toxin from the point of view of preservation by means of radiation. In this respect we can say that the toxins demand anazingly large radiation doses to be inactivated. The inactivation doses for the toxins thus seem to be of about the same magnitude as the dose which is considered necessary to destroy spores of Cl. Botulinum. It is also noted that larger doses are necessary for inactivation at quite low temperatures. The results further suggest that smaller units of the toxin molecule are not easily inactivated.

Secondly, the results of these investigations have contributed to the clarification of certain important facts with regard to the toxin itself. Moreover, there is considerable uncertainty with regard to the size of the toxic unit of the botulinum toxin. It can be advantageous to use radiation in such investigations because one is then not dependent on pure production of toxin. On many occasions such purification processes can probably cause a change in the structure of the toxin.

In summation, one can say that these investigations seem to suggest that radiation is not an especially effective method for inactivation of botulinum toxins. Heat treatment can be said to be more effective and cheaper, and will therefore continue to be the most practical treatment in most situations.

In conclusion, one can say that heat treatment is by far more effective, cheaper, and more useful.

Literature

Cardella, M. A., Duff, J. T., Gottfried, C. and Begel, J. S. 1958, J. Bact. 75, 360-365.

Duff, J., Wright, G. G. Klerer, J., Moore, D. E. and Bibler, R. H. 1957a, J. Bact. 73, 42-47.

Duff, J. T., Klerer, J., Bibler, R. H., Moore, D. E. Gottfried, C. and Wright, G. G. 1957b, J. Bact. 73, 597-601.

Lea, D. E. Actions of radiations on living cells. Cambridge University Press 2nd ed. 1955.

Reed, R. O. and Munch, H. 1938 Amer. J. Hyg. 27, 493-497.

Sterne, M. and Wentzel, L. M. 1950 J. Immunol. 65, 175-183.

Taimuty, S. I., Towle, H. L. and Peterson, L. D. 1959 Nucleonics, 17, No. 8, 103.

Wagenaar, R. O. and Dack, G. M. 1956 Food Res. 21, 226.

TYPE C-BOTULISM IN MEN AND ANIMALS

(With Special Reference to the Appearance of the Disease in Cattle and Horses)

by

J. Müller

Botulism, the characteristic poisoning illness in connection with consumption of food in which Clostridium botulinum has had opportunity to produce toxin, is fortunately not frequent among people.

The number of cases of human botulism registered in the whole world over the last 50 years was in 1956 estimated to about 5,635, i.e., only about 100 cases a year. The very dramatic and serious character of each outbreak of the illness fully explains, however, the special interest which people have always taken in this special form of food poisoning. In the above mentioned period there were 1,714 fatal cases reported among the people contaminated by botulism, which means an average mortality of about 30%. The highest mortality rate is found in the US where 67.7% of 1,350 contaminated people died; the outbreaks in Europe have, on the other hand, been of a milder character. A mortality rate which for example in Germany, varied with few exceptions between 10 and 19% and in France varied between 1.5 and 8%. (7)

As for Denmark, we have a collocation of 10 outbreaks of botulism from 1901 to 1959 involving 32 people, of whom 13 (40%) died. (4)

The interest of the canning industry in this illness is naturally due to the fact that toxic preserves have proven very often to be the direct reason for outbreaks of botulism. On the whole, botulism can break out whenever food articles which are contaminated by Cl. botulism have undergone a definite but unsatisfactory treatment (for example, by heating, salting, smoking), are kept for some time, and are then eaten without having undergone further heat treatment. The decisive factor is that Cl. botulinum spores under certain conditions can be extraordinarily thermo-resistent,

and that under suitable conditions -- especially suitable degrees of acidity (pH 4.6 - 9.0) and isolation from the oxygen of the air -- they can grow and give rise to formation of extraordinarily strong poison.

When over the years it has been possible to determine the bacteria in outbreaks of human botulism, it has as a rule been the types A, B, and E. Among these, types A and B are known to have caused botulism also in animals, often in connection with outbreaks in people and of the same origin: consumption of tainted canned food.

Types C and D which are responsible by far for the most frequent and the most serious outbreaks of botulism in animals have not, on the other hand, been considered to represent any danger to people; however, in the future it would perhaps be wise to keep this possibility in mind. Type D has certainly never been found to cause human boutlism, but from recent years we have at least two cases of type C-botulism in people, one from the US in 1953 (8) and one from France in 1955. (17)

This is the background of the survey I shall give on the topic of the appearance of C-botulism in animals, especially in horses and cattle. At the same time I shall give you the results of the investigations made in this field in Denmark over the last two or three years.

Type C-Botulism in People

The first case of mortal botulism was of uncertain alimentary origin. Dissection of the patient indicated a botulinum toxin in the stomach contents, the type of which could not immediately be determined. In cultivating the material, a toxin-producing, anaerobic bacterium which behaved like Cl. botulinum of the group C-D-E in morphological and biochemical respects and which was neutralized only by botulinum-antitoxin of type C alpha was cultivated purely . Guinea pigs immunized by anatoxin from this rod were protected against type C toxin. It was found that 42,000 lethal mouse doses did not cause illness in a kid at the time when it was fed, but that 2,000 doses, given in the same manner to a monkey, caused lethal botulism.

In the second outbreak, at Auvergne, France, two persons out of eight became ill 3 days after they had eaten a paté de campagne. They showed characteristic eye symptoms, suffered from swallowing and speaking difficulties, dryness in mouth and throat, eructation, constipation and bladder paralysis. Furthermore, they suffered from great feebleness, rapid pulse, and weakened reflexes. After 6 days in hospital during which time the two patients were treated with polyvalent vaccine since they refused for some reason to be treated with serum, they were released in improved condition but were very weak.

Toxin could not be found in three specimens of the paté de campagne. Moreover, two of the specimens were sterile, but from the third was produced in pure cultivation Cl. botulinum which by means of specific antitoxic sera

could be defined as belonging to type C beta. In a gulcose bouillon there was a toxin formation corresponding to 200,000 lethal mouse doses pr. ml. over a 6-day period. The rod was the most poisonous C-rod that had been isolated in France at that time.

Type C spores are less thermoresistent than those of types A and B. Therefore, there were probably surviving spores and toxin formation only in the middle part of the paté, and this can be the explanation why only 2 out of 8 people became ill.

We naturally know nothing about how much toxin the people in these two cases of C botulism had consumed. It is mentioned that 2,000 lethal doses (probably mouse doses) of C alpha toxin produced from the rod which was isolated in the American case were enough to kill a monkey at the time of feeding. There are earlier American tests, (3) which, among other things, compare the toxic effect of type B, C alpha-, C beta-, and D toxin on Macacus rhesus, which is biologically close to man. In these tests, two monkeys survived amounts of 2,000 and 30,000 lethal guinea pig doses of C alpha toxin consumed through the mouth, while one monkey died after a hypodermic injection of 500 doses. For comparison, it can be mentioned that only 100 doses of type B toxin given to a monkey through the mouth was enough to cause mortal botulism. The same result was reached in two monkeys which were orally given 9,450 and 24,500 doses of C beta toxin, while two monkeys survived 40,000 and 200,000 doses of D toxin given in the same manner.

It is thus very apparent that with the consumption of food the toxin of the C types is less poisonous to primates than the toxin of other types which usually have been identified in connection with human boutlism. However, as these cases show, such poisonings can occur. Since the consumed amount of toxin may be of especially great importance in the appearance of this type of botulism, it is plausible to assume that in these cases it was very toxic rods that caused the illness. The results of the investigations also suggest this.

Type C Botulism in Animals

Also with regard to botulism in animals, the poisonous situation can come about in a faster or a slower way, the reason for which is that the Cl. botulinum poison is obtained through the feed. In some cases this has been conspicuous, and therefore the illness has come to be called "forage poisoning." In other cases it has been more difficult to detect the reason of the illness as can be seen through such illness designations as "Paralysis Illness" (Lamsiekte), "Spinal Paralysis," "Enzootic Cerebrospinalmeningitis" "Enzootic 'Bulbar' Paralysis," "Limberneck." In these cases one has taken note of only the most obvious symptoms and of the fact that similar outbreaks occurred time and again in the same neighborhood, which leading to the belief that it was a contagious illness. Here, as with people, the outbreaks of botulism were blamed on the neuro-muscular apparatus.

I do not intend to tire you with long clinical descriptions of symptoms of the various kinds of spontaneous botulism in animals. For your orientation I will just mention:

As for horses, faster or slower paralysis of the limbs takes place in connection with pronounced weakness, stupor, and difficulty in breathing. These symptoms dominate the picture while paralysis of the tongue and the throat with accompanying difficulties in receiving and swallowing the fodder is less frequently noticed.

As for cattle, the symptoms of paralysis of the cerebral nerves are, on the other hand, very characteristic and draw one's attention to the possibility of botulism. Lips, tongue, lower jaw, and throat are gradually paralyzed so that eating and swallowing of the food become completely impossible. The animals stand or -- in later stages -- lie with the tongue hanging from the mouth and with the saliva driveling from the muzzle. Similar illness symptoms can be seen in sheep.

Both in horses and ruminants one can find all variations between violently developing cases with early paralysis and death, and light cases in which the animals, after having shown one or serveral of the above mentioned symptoms for some time, recover completely. The convalescence of the cows is the shortest; it can take horses months after the acute stage of the illness to be fully healthy and useful again.

There is no information of C botulism in hogs and the question whether hogs can catch the illness spontaneously at all ought to be left unanswered. The little information that is to be found in the literature is mostly of clinical origin and is not sufficiently verified by toxicological and bacteriological investigations. (11) (19)

Minks show pronounced paralysis of the limbs, great weakness, and a very characteristic, strained, and 'pumping' breath. (2a)

Dogs (14) and cats (14, 16, 13) are for all practical purposes refractory to spontaneous botulism, but there are reported cases of simple type C botulism in hunting dogs (25, 12).

Fowl, to a lesser degree cocks and hens, (1) but especially ducks and a series of wild birds (5, 20) are attacked by C botulism. They show movement disturbances, cannot fly up, and are in the end lying still on their stomachs with half-spread wings and with their heads and necks resting on the soil before them or on their backs.

The mortality of type C botulism in animals is on the whole very great and can fully be compared with that of the serious outbreaks of human botulism, and since there are so many more living creatures involved in animal outbreaks, botulism of cattle (21), sheep (22), and minks (18, 2) is of very great economic importance in certain countries.

In Australia and Tasmania the illness appears in cattle and sheep particularly in the late summer when the grass is dry and the feed scarce. (22) At this time the animals suffer from lack of phosphorus and perhaps also from lack of protein, and they try to acquire what they lack by gnawing carcasses they run across on their way. These carcasses, whether they are of cattle, sheep or rabbits, can contain large amounts of botulinum toxin. It is found out, for example, that only 3 g. of a 8-10 day old rabbit cadaver can kill a sheep due to botulism.

In South Africa they have a rather similar problem. It is reported that 100,000 cattle die yearly under similar circumstances. The lack of phosphorus in the soil and the grass causes an a-phosphorous state in the cattle which makes the animals eat the carcasses they find. In certain areas a great percentage of these carcasses is highly toxic due to Cl. botulinum. In particular, cadavers of tortoises/turtles (:skildpadder) can become extraordinarily poisonous. The South African Paralysis Illness (:Lamsiekte) is quite certainly most often caused by type D which seems to be especially widespread in Africa, but 10-20% of the cases are caused by type C. (21).

Both in Australia and South Africa we find C botulism in horses, but among them mostly in those which are fed in stables. In Australia various vegetable feeds are reported as origins of the outbreaks. However, the outbreaks are often due to badly harvested, badly kept, rodent-infested (:gnaverbefangt) feeds. From South Africa it is reported that botulism was contracted by mules due to formation of type C toxin in a rat cadaver found in the fodder manger. (24)

Similar mass outbreaks of botulism are not known in Europe with its more intensive cattle-breeding, but investigations especially during the last 10 years have revealed, however, that lesser outbreaks of type C botulism in cattle and horses are rather frequent, for example, in Belgium, France, Spain, Holland, and Germany. (26) Especially from France we have a number of investigations which in many instances reveal that formation of botulinum toxin in cadavers of cats in stored fodder has been the most probable origin of the outbreaks. (16)

Of interest are the reports from America which tell of colossal outbreaks of type C botulism among wild birds. This is the so-called "Western Duck Sickness" which occurs in shallow and alkaloid lakes and swamps in a belt over Western America from Canada to Mexico. Tens of thousands of birds, mostly ducks, are involved. In one year, 1932, the mortal cases have been estimated at about 250,000. Due to the special conditions which prevail in these areas where algae fill the shallow, slightly alkaloid water, the necessary conditions -- temperature, lack of oxygen, etc -- are established for the development of the fatal toxin.

Both in America and Europe type C botulism plays a great role in the raising of minks, because outbreaks are very violent, very rapid in their development and have extremely high mortality. It should be mentioned that in many instances it is proven that the outbreaks have been due to feeding with slaughter refuse or cadavers. (15, 2a).

Botulism In Cattle and Horses in Denmark

In the Danish veterinary law dealing with dangerous, contagious illnesses we find enumerated one "Contagious Throat Paralysis in Cattle" and one "Dangerous Spinal Marrow Typhus in Horses." The first illness has been known for at least 50 years (6) and the second for more than a 100 years. (23) The symptoms of these illnesses are much like those which were described as characteristic for botulism in cattle and horses in other countries at the beginning of this century. Therefore, it was already thought in the 30s that the two illnesses, which quite often appeared simultaneously on the same farm, should be regarded as outbreaks of botulism rather than as a manifestation of undiagnosed infectious illnesses.

At the investigations we made at the State Veterinary Serum Laboratory in Copenhagen during 1959-60 in cooperation with the Veterinary Directorate and the practising veterinarians we found proof of the fact that the cause of most outbreaks of these illnesses has been botulism of type C. (9)

On the basis of H. Pedersen's observation (10) in connection with investigation of type E botulism that mice which have died from toxic feed are the place of rapid formation of Cl. botulinum with accompanying toxin growth in the mice's liver, we have obtained from cows and horses which have died from throat paralysis and spinal marrow typhus at least a big piece of liver for investigation.

We extracted the liver pieces with isotonic buffers at pH 7.3, in such a manner as to use equal layers of liver and "push cushions." We then injected the centrifugalized extract, after adding to it penicillin and streptomocin, intraperitoneally into white mice in a dose of 1 ml. Heraby on a number of occasions we were able to point out botulinum toxin in varying, but often considerable amounts (up to about 100,000 lethal mouse doses pr. ml. 50% liver extract). The titrated toxic extracts have been subjected to neutralization attempts with specific antitoxic sera of types A, B, C, D, and E, from Institut Pasteur in Paris, and all toxins investigated so far have easily been determined as type C.

We also always set up cultures from the liver pieces in 300 ml. thoroughly boiled 0.2% glucose solution (Inst. Pasteur) covered with paraffin oil, and we incubated these cultures at 37° for 4-8 days, partly without further heating, partly after heating in water at 60° for 60 minutes. In this manner, we were successful in ascertaining the presence of Cl. botulinum

in the toxic cow and horse livers, since we were able to achieve strong toxic cultures. The neutralization attempts undertaken so far have only revealed the presence of the botulinum toxin of the C type in these cultures.

All in all, we have until now pointed out botulinum toxin in the liver of one or several animals from 10 out of 12 outbreaks of so-called contagious throat paralysis in cows and from 3 out of 5 outbreaks of so-called dangerous spinal marrow typhus in horses.

Moreover, we have made the characteristic symptoms appear in a cow weighing about 600 kilos through feeding with toxic suspension of liver tissue (:toksisk suspension of levervåv) taken from cows which had been involved in spontaneous outbreaks. The suspension contained type C toxin of the magnitude of 500,000 lethal mouse doses. After an incubation period of 45 minutes the symptoms of poisoning began to appear, and over another 41 hours progressed to death. A two-year old horse that was given a 50 ml sterile philtrate from a toxic liver culture by means of inoculation into his stomach was sick already after 18 hours and dead after another 4 hours. It had been given about 1,000,000 lethal mouse doses -- i.e., twice as much toxin as the cow--which explains the rapid progress, which, however, is in no way unknown in cases of spontaneous botulism involving horses.

In our investigation of these two experimentally-poisoned animals which took place shortly after their deaths, there were only faint traces of botulinum toxin found in the contents of the duodenum and the liver. The positive results in this respect when it comes to cases from practical life are probably due to the fact that Cl. botulinum, whose presence in the liver of animals killed in spontaneous outbreaks we have as a rule been able to point out, has had opportunity to grow in the cadavers and produce toxin in greater or smaller amounts before the investigation took place -- perhaps a number of days after the animal's death.

Thus, botulinum toxin and the presence of Cl. botulinum were pointed out in 18 livers out of 23 from cows and horses which had died in verified outbreaks of boutlism; only toxin was pointed out in one case; only Cl. boutlinum in one case, and neither toxin nor Cl. botulinum in 3 cases.

We investigated an equal amount of cow and horse livers from outbreaks which were at first regarded as throat paralysis or spinal marrow typhus, but which later turned out to be something completely different or which did not correspond to the classic descriptions of these illnesses. In these livers we have not been able to point out botulinum toxin in extracts, nor Cl. botulinum in anaerobic cultures. Therefore, we consider our investigations to be of diagnostic significance, even if we have not yet been able to account for how the separate outbreaks of botulism have occurred.

We have also encountered information about finds of dead rats and cats in the fodder on farms where the illness occurred, but we have not been able to point out Cl. botulinum or its toxin in the few cadavers we have so far examined. Nor have we obtained any positive results from the investigations of various vegetable feeds which were blamed.

I have found in the literature only one mention of the discovery of spores of Cl. botulinum, type C, in soil specimens. In that instance it was found in 5 specimens out of 62. However, it may be assumed that in areas where C botulism occurs in animals, the spores must also be spread in the soil and may by chance be ingested and exist in the alimentary canal of domestic animals. We hope to have the opportunity to study this matter more closely.

In order that botulism shall occur, it is necessary, however, that a certain amount of toxin, which differs in accordance with each animal's sensitivity, be ingested. Whether this toxin formation can take place directly in various crops, or whether the presence of animal cadavers from which the vegetable fodders are contaminated indirectly through the diffusion of the toxin is necessary, we do not know yet. It is natural that the C type finds good opportunities for toxin growth in the presence of animal protein.

One can assume that meat and meat products are occasionally tainted by spores of Cl. botulinum, type C. But since there are still only these few mentions of type C botulism in people, it can -- as I have said earlier -- be blamed upon the fact that type C toxin is only poisonous to people in very great doses, and that the type C spores have relatively little heat resistance. In connection with our investigations we have so far been able to ascertain that they survive heating up to 60° for one hour, but that in the usually impure cultures we have dealt with, they are destroyed when heated to 100° for 10-15 minutes. Type C toxin is already destroyed after five minutes' heating up to 60°, but in the usually impure cultures with which we have dealt they are destroyed when heated to 100° for 10-15 minutes. Type C toxin is destroyed already after five minutes' heating to 80° C.

Bibliography

1. Bengtson, I. A. 1922. Preliminary note on a toxin-producing anaerobe isolated from the larvae of Lucilia caesar. - Publ. Hlth Rep. (Wash.) 37, 164-170.

2. Dinter, Z. & K. E. Kull. 1955. Botulism in Mink - D.T.W. 62, 53-55.

2a. Dinter, Z. & K. E. Kull. 1950. Botulism in Mink in Sweden. Nord. Vet. Med. 2, 286-301.

3. Gunnison, J. B. & K. F. Meyer. 1930. Susceptibility of monkey, goats and small animals to oral administration of botulinum toxin, types B, C and D. - J. inf. Dis. 46, 335-340.

4. Jensen, B. Bang & F. Hahnemann. 1959. Boutlisme. Two Outbreaks Which Developed Much in the Same Manner - U.f.L., 121, 1363-1368.

5. Kalmbach, E.R. 1934. Western Duck Sickness: a form of botulism - Techn. Bull. No. 411, U.S. Dep. Agric (Wash).

6. Kristensen, Markus. 1912. Observation of Epizootic Throat Paralysis of Cows and the so-called Spinal Marrow Typhus of Horses, Veterinarian, monthly, 23, 545.

7. Meyer, K. F. 1956. The status of botulism as a world health problem. - Bull. W.H.O. 15,281-298.

8. Meyer, K.F., B. Eddie, G.K. York, C.P. Collier & Ch. T. Townsend. 1953 Clostridium botulinum type C and human botulism. - Congr. Internaz. Microbiol., Roma, Riass. delle Comm. Vol. II, p. 276.

9. Muller, J. 1960. Botulism in Cattle and Horses, Journal, Danish Veterinary Society, 43, 791-796.

10. Pedersen, H. O. 1953. Botulism - Nord. Vet. Med. 5, 700-730

11. Petersen, P.C. 1957. Botulism in Swine, Journal, Dan Vet Soc 40, 69-72.

12. Pilet, Ch. H. Cazabat & R. Ardonceau. 1959. A New Botulism Disease in Hunting Dogs, - Bull. Acad. Vet. France. 32, 297-303.

13. Prevot, A. R. & E. R. Brygoo. 1950. Research on Toxin, Anatoxin, and Antitoxin of Botulism D. - Ann. Inst. Past. 79, 1.

14. Prevot. A. R. & E. R. Brygoo & R Sillioc. 1953. New Research on Botulism and its five Types of Toxin. - Ann. Inst. Past. 85, 544-575.

15. Prevot, A. R. P. Collin & R. Sillioc. 1956. Study on Mink Botulism in France - Rec. Med. Vet. 132, 188-193.

16. Prevot. A. R. & R. Sillioc 1955. A biological Enigman: Cats and Botulism - Ann. Inst. Past. 89, 354-357.

17. Prevot. A. R., J. Terasse, J. Daumail, M. Cavaroc, J. Riol & R Sillioc Human Botulism, Type C, in France, - Bull. Acad. Nat. Med. 139, 355-358.

18. Quortrup, E. R. & J. R. Gorham. 1949. Susceptibility of furbearing animals to the toxins of Clostridium botulinum, types A B, C and E. - Amer. J. Vet. Res. 10, 268-271

19. Scheibner, G. 1955. The Susceptibility of Hogs to Botulism, Type A-E, D.T. W. 62, 355-358.

20. Robinson, E. M. 1930 The bacteria of the Cl. botulinum C and D types. - 16. Rep. Dir. Vet. Serv. Anim. Ind. (Union South Afr.) 107-142

21. Sterne, M. & L. M. Wentzel. 1949. Botulism in animals in South Africa. - 14. Internat. Vet. Congr. London. Vol 3, 329-331.

22. Seddon, H. R. 1953. Diseases of domestic animals in Australia, Part 5 Vol 1; Bacterial Diseases, p. 82-99. - Service Publication (Div. Vet. Hyg.) No. 9 (Sydney).

23. Stockfleth, H. V. 1859. How is the disease called Spinal Marrow Paralysis, or rethare, Spinal Marrow Typhus in Horse recognized? -- Tidsskr. f. Vet. 7, 237-254.

24. Theiler, A. & E. M. Robinson. 1927. Botulism in Domestic Animals. Z. Infekt Kr. Haustiere, 31, 165-220.

25. Vallee A., A. Kreuger & A. Eyguem. 1956. Botulism in Dogs; First Source Isolated in France. - Bull. Acad. Vet. France. 29, 419-423.

26. Verge, J. 1951. Animal Toxin Infections and Alimentation. - Rec. Med. Vet. 127, 767-828.

Environmental Conditions of Growth and Toxin Formation of Cl. botulinum with Special Regard to Vacuum-Packed Foods

By A. Johannsen

Table of Contents

INTRODUCTION

Cl. botulinum has since long been classified as an obligate anaerobic bacterium that for its growth and toxin formation demands absolute absence of the oxygen of the air. The removal of air, according to public opinion, creates conditions of growth and toxin formation, for example, in vacuum-packed foods. The modern view is, however, that this is not the case since the metabolism of the bacteria, like that of other living cells, is regulated by complex systems whose activities are dependent on many factors, such as temperature, pH and buffering ability, partial oxygen pressure, red-oxpotential, steam pressure, synergetic or antagonistic factors, antibiotic substances, necessary biological systems with regard to enzymatic effect, etc.

In order that the bacterium shall be able to grow and germinate toxin, all these conditional factors must be concentrated in an area which is limited in various respects by the tolerance of the bacterium. Experience also shows that these limits are relatively narrow, since the appearance of botulism must be regarded as rare, although the bacterium is common in nature and perhaps more often than one thinks enters foods as impurity or perhaps mostly as natural infection in various raw substances. In most cases there ought perhaps also to be a minimum infectional amount for the bacterium to grow, an amount which is influenced by the preparation and treatment with heat, oxygen, acid, salt, smoking, etc., of the product.

In order to find out the possibilities of growth and toxin formation of Cl. botulinum in various foods during changing conditions, extensive biological investigations and experiments must be undertaken. Such investigations and experiments comprise a searching for empirical facts, which more or less constitute substitutes for lacking biochemical knowledge. The biochemical processes which according to plan in each single case regulate metabolism, growth, toxin formation, and other life functions of Cl. botulinum are, however, of such complicated and varying nature that comprehensive and complete biochemical knowledge is impossible. Factors which are of essential importance for the growth of Cl. botulinum in a certain product are, among others, the chemical composition of the product, its physical condition, and the way it is prepared.

LITERATURE

The Chemical Composition of the Product

The chemical composition is of importance in the first place in regard to the existence of the general and specific nutrients which are necessary for the growth. It should here be observed that the nutritious need can vary with the conditions under which the growth occurs. It is thus possible that Cl. botulinum can grow under seemingly aerobic conditions in case the product contains partly specific nutrients and partly such substances which either directly or through the influence of other microorganisms can establish the conditions which Cl. botulinum demands.

How the bacterium influences and utilizes carbohydrate, fat, and albumen is not known in detail. The information in the literature is often contradictory. We know, however, that carbohydrates hurry the growth but slow down the spore formation. During the fermentation of carbohydrates, intermediary and end products can create favorable growth conditions for Cl. botulinum, but, on the other hand, the acid formation at such times can be so great that growth and toxin formation become impossible. Furthermore, the presence of carbohydrates creates favorable conditions of growth for microorganisms with antagonistic or antibiotic influence.

The protein structure of foods varies greatly and is complex. Different microorganisms demand different amino acids, which are also attacked in various ways. The possibility of growth of Cl. botulinum can exist due to various synergetic circumstances such as symbiosis with other microorganisms. It is considered that most rods of Cl. botulinum demand three amino acids: l-alanine, l-phenylanine, and arginine. In a solution containing 5% digested meat and 0.05% natriumthioglycolate, 94-99% of Cl. roseum and Cl. botulinum, type B, germinate within 15 minutes. Other amino acids slow down the germination process (Halvorson).

It has not been determined whether the Cl. botulinum bacteria, of which part are proteolytic, can utilize complex proteins for their growth when there is no other nitrogen source. The proteolytic rods reveal themselves through changes in the substrate in which they grow, among other things, through a distinctive putrid smell. The rods which are not proteolytic show no change worth mentioning and never have any bad smell. All C, D, and E rods are regarded to be non-proteolytic. Zeller (1959) gives types C, D, and E as animal-pathogenic and proteolytic, and A and B as mainly human-pathogenic and not proteolytic. The concept of proteolysis is limited to hydrolysis, which leads to formation of peptides and amino acids. Many of the anaerobic bacteria are especially active in the breaking down of complex proteins; the method of splitting has been studied by several scientists. The hydrolytic breaking down is dependent on influence from special enzymes formed by these bacteria. During their

growth they are able to produce a sufficient number of enzymes to start the protein splitting and thereby to increase the amount of available nitrogen.

On the whole, bacteria utilize amino acids in various ways, but the utilization is more dependent upon the kind and condition of the bacterium than upon the nature of the nitrogen composite that is attacked. Decarboxylation, deamination, and transamination are essential processes in the breaking down of amino acids. The enzymes which cause these processes are relatively specific for each amino acid. At the breakdown, among other things, pyridoxal phosphate, a necessary growth factor for Clostridia, is formed. Certain Clostridia lack the ability to utilize carbohydrate to a greater extent, and are dependent on amino acids for energy.

It is generally thought that the supply of fermentable carbohydrates saves amino acid, peptides, or protein in a medium. This effect can, however, be illusory, and is mainly a fermentative process. The low pH-value caused by fermentation of carbohydrate prevents or stops the growth or checks the decarboxylation enzymes. The carbohydrates can also cause changes in the enzyme constitution of the bacteria, in this manner influencing the nitrogen metabolism, which decreases the use of nitrogen material as energy source.

With regard to bacteria generally, and especially with regard to Cl. botulinum, our knowledge of protein metabolism is limited to relatively simple systems, and much here is still shrouded in darkness. The process, which is complicated even in simple systems, naturally becomes very difficult to comprehend when the substrate consists of complex foods.

Theoretically, the use of synthetic media offers a method to establish the more precise importance of various nutritious factors like organic acids, amino acids, growth factors, etc., with regard to spore formation by bacteria. In practice, however, such media have not been used, especially due to poor spore formation or completely lacking spore formation, although good vegetative growth was obtained. Synthetic media, especially with regard to anaerobes, have not been greatly used, and such a medium for Cl. botulinum has not existed for many years. By using such a medium, investigations have, however, been made of the effect of some organic substances upon sporulation of Cl. botulinum type A. Thereby it is found out that an admixture of glycine, ornithine, arginine, beta-alanine, creatine, sodium butyrate, and sodium valerate has a stimulating effect. A lesser effect was obtained with proline, asparagine, and glutamine. No stimulation was obtained by glutamine acid, cystine, coline, norleucine, or lysine. Dextrose stimulated the growth markedly but had a strong represent effect on the sporulation (Williams, Blair).

It is an old opinion that the presence of dextrose in a nutrient decreases spore and toxin formation. Gibbs and Hirsch (1956) examined this problem in connection with usage of RCM (Reinforced Clostridial Medium) and

found thereby that types A and B of Cl. botulinum behaved differently. Type A was not influenced in its spore formation through dextrose concentration in the medium, while type B showed a higher proportion of spores at the lower concentration than at the higher (0.1 and 2%, resp.).

Both types showed higher toxin production in the higher dextrose concentration (10^3 and 10^2 in connection with injection of 0.1 ml in the highest 10-fold dilution of the culture).

According to Zeller (1959) cultivation in dextrose broth (type B) constitutes a diagnostic venture, since it has been proven that dextrose in a nutrient causes a rapid and complete breakdown of the bacteria and prevents spore formation. It is therefore impossible to get sub-cultures. Cl. botulinum has, on the whole, a pronounced tendency to break down, which naturally becomes much more apparent in dextrose-containing media.

An admixture of 0.12 - 1% of sorbic acid at pH 5.6 to the usual culture media makes all types of Cl. botulinum grow rapidly and form toxin (York, Vaughn).

When it comes to essential nutrients and environmental conditions necessary to the growth and toxin formation of Cl. botulinum in foods, much information is obtained from recorded outbreaks of botulism. During the 18th and 19th centuries there were outbreaks of botulism in Germany caused by smoked bloodsausage, liver sausage, smoked pork, smoked ham, rotten cheese, etc. From Russia there are cases reported which were usually caused by various kinds of fish such as salmon, sturgeon, herring, and others, while in the US most cases have been caused by bad canned vegetables, olives, and spinach, but also by imported canned sardines and smoked salmon. From the Nordic countries cases are reported caused by fish such as mackerel, canned salmon, salted herring, but also by meat-balls, home-cooked braunschweiger, and salted ham.

All these products have such qualities and such essential amino acids that under certain conditions growth and toxin production of Cl. botulinum is possible.

The pH-value

A highly essential factor, when it comes to environmental conditions of Cl. botulinum, is the pH-value and the buffering ability of the product. Growth is not possible at pH-values below 4.5 (Ingram, Robinson), a condition which is of practical importance in the sterilizing of acid products. H. O. Pedersen found a pH-value of 4.6 in marinated herring that had caused a case of poisoning. The border-line ought therefore to be very narrow. In experiments with herring marinated for 24 hours in strong vinegar with a pH 2.9-value, Pedersen found no reduction in growth or toxin production, due to the buffering effect of the muscles, which after 6 days showed pH 6.6. In an outbreak of botulism caused by home-cooked spiced herring which

was investigated by the Board of Health in Lund, the herring in question showed in a 10% suspension in a physiological common salt solution a pH-value of 5.8 and a toxin proportion of between 10-100 MLD/g. Thus when the buffering ability is high, changes in the micro-flora of the product are not easily brought about. When it is low, the normal micro-flora of the product determines the pH-value of the product. Thus, one can often find in stored products pH-values which are remarkably low due to the tendency of the initial flora in the direction of acid-forming micro-organisms. A strong buffering ability in the product is thus to be regarded as a favorable environmental condition of growth of Cl. botulinum.

In the cases when toxin is pointed out in a product with a pH-value under 4.5, it is apparent that the toxin was formed before this pH-value was reached. This can be due to (1) the fact that the diffusion of acid is slow, (2) the fact that the acid was produced through growth of Cl. botulinum of (3) due to other acid-producing microorganisms (Ingram, Robinson).

The lower the pH-value, the shorter to the time demanded for destruction of spores by heating. Spores are killed by 10% hydrochloric acid during 1 hour at room temperature (Dazier). At least 2% vinegar in the marinade is necessary to kill the spores in marinated products. High pH-values around pH 9, as for example in chicken egg whites, generally represent an obstacle to bacterial growth. The highest pH-value which allows the growth of Cl. botulinum is not given in the literature.

The Red-oxpotential

The spores of anaerobic bacteria cannot grow if the red-oxpotential in the product surpasses a certain definite value (+140 mV for Cl. sporogenes, +60 mV for Cl. histolyticum, at pH 7.0). The red-oxpotential is dependent upon the balance between oxidizing and reducing systems. In fresh products the potential is as low as in living cells.

The red-oxpotential in a food is in practice determined by:

1. the characteristics of the product
2. access of oxygen.

The SH-groups in animal tissue and ascorbic acid and reducing sugar carbohydrates in plant tissue give a low red-oxpotential. The balance capacity is represented by the ability in the system to resist changes from outside, in this context the oxygen of the air. It seems to be high in "living" foods which have kept their cellular respiration. Meat, for example, can maintain a low red-oxpotential and use up considerable amounts of oxygen; even bacon keeps this ability to a certain extent. The effect of the oxygen only reaches a few mm. from the surface, which can be seen from the superficial oxidation of myoglobin. Anaerobic bacteria can therefore grow in meat irrespective of the access of air, i.e., sufficiently

anaerobic conditions exist in less permeable parts of the product or in the center of bigger pieces (Ingram, Robinson). The resistance to oxidation in such products depends on the activities of natural reducing systems located in the tissue structure, which, however, is easily damaged by heat and chemicals. The reducing systems are, for example, destroyed to a great extent in cooking and pasteurizing. In roughly treated products the regeneration of the balance system is prevented, so that the potential is rapidly increased when the product is exposed to the oxygen of the air.

For growth of Cl. botulinum, the oxygen level cannot surpass 1.14 - 1.36 cm Hg or 7.2 - 8.6% of atmospheric oxygen. The micro-flora of the product can influence the red-oxpotential in one or the other direction. Thus, it is considered that certain coliform bacteria and certain lactoacid bacteria can lower the red-oxpotential so that spore germination of anaerobic bacteria can occur. In other foods, on the other hand, with a natural bacterial flora which influences the raising of the red-oxpotential, this can -- in otherwise apparently anaerobic conditions -- make the growth and toxin production of, for example, Cl. botulinum impossible.

The product can in itself contain chemical components which exercise a more or less repressing influence upon growth, spore-, and toxin-formation in various kinds of food. Certain fats which undergo autooxidation, special proteins such as lysozym in eggs and lactenine in milk, and also a number of other substances have, on the whole, an anti-microbial effect. Such substances seem also to appear through storing and heating.

Through oxidation of fatty acids, organic peroxides are formed that at their breakdown can form H_2O_2, which, as we know, generally has a strongly repressing effect on Clostridia. Unsaturated fatty acids and their methylesters cannot by themselves prevent spore germination and growth except in rancid conditions. It is therefore probable that at their oxidation a product with repressing effect appears; it can then in the first place be H_2O_2 since catalase partly reduces this ability. It is known that soluble starch counteracts the repressing effect of the peroxides of the fatty acids (Olsen, Scott, 1946).

The Physical Situation

Many times the physical situation of the product has a decisive effect upon the environmental conditions of Cl. botulinum. The effect of the frequently heterogenous composition of the product is that the physical situation of one or several components determines whether or not the environment is favorable. Factors which regulate the physical situation are: freezing, changes of the structure, emulsion situation, changes of colloidal components, steam pressure, etc.

It is considered that microbial activities are wholly stopped when freezing takes place, even if the enzyme activities are not fully inactivated. But freezing is a process that takes place gradually, even if one strives

to do it quickly. During the freezing process the water of the product is frozen first and is distinguished as ice; thereby the concentration of dissolved components increases, and the freezing point is lowered. The freezing is not finished until a sufficiently large amount of water is separated. There is thus a temperature at which the product seems frozen but contains an amount of water whose concentration depends on the temperature. If the freezing is done quickly, the ice is separated into small crystals, which, however, seem considerably bigger if the freezing is done slowly. In either case, a division of the water of the product has taken place which can give rise to changes in the product when it is thawed, and, further, the normal cellular walls have more or less broken down. When the product is thawed, the water situation is more or less changed; this, together with the structural changes in the product, can have effects in one way or another on the growth conditions of Cl. botulinum. Among other things, one can imagine decreased resistance to infection. Common rancidity bacteria are mainly limited to the water between the muscle fibrils. Growth and toxin formation of Cl. botulinum during the freezing and thawing processes have not been noticed (Saleh, Ordal). The quick growth of anaerobic bacteria in meat, especially in whale meat, does not take place until after rigor mortis, which causes micro-structural changes in the muscles. Before rigor mortis, most dissolved components are within the muscle fibrils. Proteins and starch most often resist microbial attacks better in native than in coagulated form. The coagulation of proteins and the gel-formation of starch imply structural changes which are of importance for the environmental conditions. It is not only the effect of the colloid which is in itself of importance, but also that of the released inhibited water which is one of the reasons why cooked products are generally more susceptible than correspondong fresh products.

A water proportion in the substrate of at least 30% (Harmsen) is necessary for the growth of Cl. botulinum. Type A does not produce toxin even after an incubation period of 9 months at 37° C in material with less than 30% water.

The activity of water in a product determines whether Cl. botulinum can grow under existing conditions. Usually we speak about water proportion, but the essential thing is how and in what way the water is bound to the dry substance. We also speak about equilibrium relative humidity, e. r. h.. The equilibrium relative humidity is apparently a factor which directly determines the kind of microbes that exist in a product. Bacteria grow only at rather high degrees of humidity, in which situation free, condensed humidity on the surface is favorable to the spread of actively mobile species. The situation is complicated due to the activities of various microbes either through formation of metabolic water or through changes in the nature of the substrate. Fatty substances have no capacity to bind water worth mentioning. Starch binds water firmly, and the equilibrium relative humidity becomes low. Cl. botulinum may demand a rather high equilibrium relative humidity in the product for its growth. In experiments it is discovered that growth and spore production is normal

at a water proportion of 60% (e.r.h. 96 - 98%). At 45% (e.r.h. 94 - 96%) both growth and toxin production is influenced in a negative direction, but does continue. At 40% (e.r.h. 90 - 95%) sparse growth can occur with or without sporulation, but usually there is no growth. Spore production occurs at all degrees of humidity (Williams, Purnell).

Changes in the Product Through Treatment

By long experience we have acquired knowledge about changes in the product which make it stable and safe. This knowledge does not concern the changes as such but primarily its consequences. From the earliest times even primitive peoples have used various methods to prepare, store and preserve foods. Changes which bear upon the product itself are primarily changes in the water ratio (drying), pH (marinating and other sour pickling, admixture of tartaric acid or sour salts), admixture of preservatives (sulphur dioxide, benzoic acid, etc.). In certain traditional processes various effects and changes are combined. Thus, salting and smoking are used both for dehydration and preservation.

Apart from drying, the water ratio is lowered through salting and marinating in sugar. van Ermengen pointed out the necessity of using a strong curing brine when curing pork, since the salt is unfavorable to growth. He made use of this fact for diagnostic purposes, since 5-6% common salt in the medium completely prevented growth. Toxin can, however, be pointed out in broth containing 5% common salt or more.

In salted meat inoculated with Cl. botulinum, toxin formation without organoleptically noticeable changes was observed when the product contained 6.25, 7.09, and 7.12% salt. A piece of leg of pork treated so that it would contain 5.5% salt showed in analysis considerable variations in this respect at the end of the salting period. Forty-nine percent contained salt in concentrations between 6.10 and 8.35%. Possibility of toxin production without organoleptic changes does exist then, and the product constitutes a hygienic risk. The salt content must therefore be adjusted in such a manner that possible growth and toxin formation of Cl. botulinum are connected with apparent organoleptic changes (under 6.25%) or must be so high (more than 9%) that toxin production is completely out of the question (Greenberg, Silliker, Fatta).

It is thought that in salted fish the salt content should not be lower than 10%, but usually a salt content of 8% is enough to repress growth.

After inoculation and incubation of surface-ripened cheese, type A spores could grow and produce toxin in salt concentrations up to more than 8%, while type B spores could not fully tolerate 6%. Fully ripened cheese was a medium inferior to moderately ripened cheese (Wagenaar, Dack).

Concentrations of $NaNO_3$ from 0.05888 to 0.392% do not prevent growth of Cl. botulinum, and concentrations of 2.23 - 4.427% do not give complete prevention. Commercial salt mixtures (NaCl, $NaNO_3$, $NaNO_2$) are effective in preventing growth and toxin production of Cl. botulinum when the proportions of the mixture with regard to the medium reaches the critical concentration of NaCl (Tanner, Evans). Yesair, Cameron (1942) found that an amount of 0.1% $NaNO_3$ and 0.005% $NaNO_2$ in meat agar resulted in a spore reduction of about 70%. Corresponding reduction took place at 2% NaCl. When 3.69% NaCl was used, the reduction exceeded 99%. This corresponds to the minimum concentrations of the allowed salts ($NaNO_2$, $NaNO_3$, and NaCl) and was introduced as a control of total salt concentrations. Thus there seems to be a pronounced repressant effect characteristic especially of $NaNO_2$; however, at the maximum concentration of NaCl (3.69%) all show complete or almost complete repression. The salts $NaNO_2$, $NaNO_3$, and NaCl exercise a repressant effect in varying degrees, individually or together. In practice, the salt combination represents a 100% repression of growth of Cl. botulinum.

In experiments with infected braunschweiger, no toxin production occurred for 9 days when commercial amounts or salt, spices, and salpetre were used, but it occurred definitely after 16-23 days. When admixing nitrite in the amounts of 50-200 ppm to the commercial mixture, the toxin production was delayed with the greater amounts. When the salt concentration was increased to 3%, toxin production only occurred after 30 days, while 3.5% salt completely prevented toxin production (Steinke, Foster).

Among the various repressant factors -- pH, nitrite, nitrate, and common salt -- there is really no one alone that represses growth. Concentrations of NaCl (in leg of pork) ought to be considered in relation to the activity of the water.

Strong sugar solutions are bacteriostatic, and combinations of fruit acid and sugar can completely repress bacterial growth. Dextrose and fructose are more effective than saccharose and lactose. The effect is in reverse proportion to the molecule weights. High molecule weights have lesser effect; low molecule weights have greater effects.

The pH-value is lowered by admixture of acids (acetic acid, tartaric acid) and also by biologically-produced acids during fermentation (yoghurt, sauerkraut). In this manner, pH-values under 4.5 are easily attained. Regarding such products, which cannot be heat-sterilized, it is of importance that Cl. botulinum cannot grow or produce toxin in acidic medium with pH under 4.5.

The pH-value of 4.6 is often used as maximum for control of products products in which the acidic content is decisive in the prevention of the growth of Cl. botulinum. The difference is acidic tolerance with regard to various types of micro-organisms has proven to be insignificant.

The absence of gaseous formation or the repression of gaseous formation does not mean absence and repression of growth. To prevent both growth and gaseous formation it is often necessary to use different concentrations of repressing substance or repressing factor. It has been noticed that gaseous formation is prevented by lower concentrations of nisine, H_2O_2, etc., or at a pH-value which is above that which permits growth and toxin formation.

Toxin formation can thus take place at a somewhat lower pH-value than gaseous formation, which means that the absence of gaseous formation is no safe criterion. Ingram, Robinson made experiments with cultures in buffered broth. In these experiments, they found growth and toxin production under pH 5.0 only in 9 cases and at pH 4.8 only on one occasion. In this respect, any significant difference between spores and vegetative cells does not exist. The pH of maximal toxin production seems to be between 6.2 and 5.7 (McKee, Bell, Hoyer (1958)).

A low pH-value obtained with air admixture of acetic acid has proven to be more toxic to bacteria than pH-values obtained by lactic acid or hydrochloric acid (Levine, Fellers). Admixture of sugar or salt has no significant effect. Acetic acid prevents bacterial growth in almost direct proportion to the present amount. It seems as if the bactericide effect of weaker organic acids not only depends on cations but also on the fact that also undissociated molecules are active. The hydrogen ion concentration is never solely responsible for the activity. The effect of highly dissociated, unorganic acids depends chiefly on tne hydrogen ion concentration, while organic acids have a germicidal and antiseptic effect which is not in proportion to the produced hydrogen ion concentration.

The germicidal effect of acids descends in the following order: acetic acid - citric acid - lactic acid - malic acid - tartaric acid - hydrochlorid acid. The antiseptic effect descends in this order: acetic acid - lactic acid - citric acid - malic acid - tartaric acid - hydrochlorid acid.

Citric acid and lactic acid thus change place in antiseptic and germicide effect.

When cheese is ripening (Liederkranz) at 2-4°C, fatty acids are released. The acetic acid content remains relatively constant, and the propionic acid content increases less than the butyric acid content. Cheese must be stored for at least 2 months in order not to permit growth and toxin formation of admixed spores of Cl. botulinum (Grecz, Wagenaar, Dack).

Sorbic acid, propionic acid, and caproic acid are similar since they neither prevent nor stimulate the growth of Cl. botulinum, types A and B. Sorbic acid is recommended for its antimycotic affect, but does not prevent the growth of catalase-negative micro-organisms (Hansen, 11 Appleman).

Benzoic acid has long been used as a preservative in various foods. At pH 7 the presence of 3% of benzoic acid is necessary to prevent growth, while at pH 4.5 only 0.1% is necessary for the same effect. Sublethal concentrations of toxic substances often have a stimulating effect. When the pH is lowered to 3, only 0.06% sodium benzoate is needed. An increase in acidity from pH 7 to pH 3 decreases the necessary amount of sodium benzoate by more than 50 times.

The hydrogen ion concentration has the same effect in various other preservatives. At pH 7, for example, the need for sodium salicylate is 150 times greater than at pH 2.5. Sulphur dioxide is more than 25 times more effective in an acidic medium, and acetic acid about 5 times more effective in the same medium. Common salt and formalin are slightly influenced within great variations of hydrogen ion concentration.

It is apparent from what is said above that organic acids as preservatives cannot greatly prevent growth of Cl. botulinum in slightly acidic, neutral, or alkaline foods.

Freely active chlorine has a detoxifying effect on the botulinus toxin. Conventional chlorine concentrations can inactivate highly purified botulinus A toxin, but organic material counteracts or prevents the inactivation.

Cooking causes coagulation of albumen. As mentioned above, this implies structural and biological changes important to the growth of Cl. botulinum. Cooked foods are generally more susceptible than fresh foods to micro-biological attacks. The cellular membranes in coagulated form produce poorer conditions of diffusion and osmosis; this affects the equilibrium relative humidity or the biological activity of the water. Therefore, cooking has to some extent the same effect as drying. Cooking can in other cases have the effect that the essential nutrients that the organism requires are more easily released. It is practically impossible to make Cl. botulinum grow on cooked shrimp, while it grows as well on cooked herring as on fresh. If cooked shrimp is ground, a comparably dry mass is obtained, while ground herring has the consistency of thicker or thinner gruel. Cl. botulinum cannot grow in ground cooked shrimp unless the shrimp is very finely cut and water is added to obtain looser consistency.

The optimum temperature for Cl. botulinum lies between 20-30° C, but the growth is more rapid at 37° C and can at times take place at temperatures up to 45° C. The optimal temperature for toxin production is reported to be between 25-28° C. In this context, we must underscore the importance from a practical, diagnostic point of view of the investigations made by H. O. Pedersen regarding the toxin production of Cl. botulinum, type E, at various temperatures, and the apparent fact that the toxin from even strongly toxic cultures almost completely disappears after 8-10 days at 37° C, while the toxin concentration remains constant for a very long time at 30° C. Ohye and Scott have likewise pointed out that cultures at 37° C in most cases do not become toxic.

Growth and toxin production can occur at temperatures as low as 5-10° C. The maximal, optimal, and minimal temperatures with regard to type E are many degrees lower than those of A and B rods (Ohye, Scott).

Cooking and heating to 70° C inactivate the toxin of all types of Cl. botulinum. In experiments with type A toxin, the destruction was 100 times faster at 80° C than at 70° C. Dissolved substances in the product can have a protective effect on the toxin, and the heat resistance of the toxin thus varies with the consistency of the product. Type B toxin is destroyed faster than type A (Scott, Stewart).

Spores of type E are killed easily when heated at 100° C for 2 minutes or at 80° C for 6 minutes. Certain rods are reported to endure 100° C for 30 minutes. The spores from H. O. Pedersen's "Fredriksbergsstam" resisted 80° C for 10 minutes, but not for 15 minutes. The heat resistance of type E spores is reported (Ohye, Scott) to be only 1/1000 of that of types A, B, and C, which can endure cooking for 3 and 4 hours and which have to be heated at 105° C for at least 100 minutes to be killed. It is reported that spores of certain rods can endure cooking up to 22 hours. Heating at 120° C for 20 minutes is considered to be satisfactory in most cases. The lethal temperature is rather variable for different rods, and the pH-value is of essential importance for the effect of the heat treatment. The more acidic the product, the shorter is the period of destruction. Presence of common salt reduces the period of sterilization in relation to the salt concentration.

It is known that many different factors have an effect on the heat resistance, such as pH, water content, and salt content. Since earlier times it has also been known that unknown factors in products have an effect on the heat resistance in Cl. botulinum. Presence of certain vitamins can partly explain variations in the lethal temperature. Presence of ascorbic acid increases the lethal temperature, which is not directly due to its reducing effect. When synthetic K vitamin is present, the lethal temperature is significantly reduced (Reynolds, Lichtenstein).

In products with pH-values of 4.5 and below, most micro-organisms are already killed in the pasteurizing process at 70-95° C, and the acidic level prevents further growth.

Vegetables, fish, and meat are more difficult to sterilize. So-called commercial sterility is here satisfactory in preservation of food, i.e., prevention of growth of human-pathogenic microorganisms and retention of quality under certain storage conditions.

The heat treatment needed to sterilize a product most often damages the natural stability through the breakdown of its biological structure, which necessitates a complete and continual protection against renewed infection.

Certain canned foods -- for example, canned leg of pork -- are only pasturized. The development of Cl. botulinum in such products is fully dependent upon the quality and nature of the product itself. If it has a sufficiently low pH-value, and suitable salt concentrations, etc., no growth will occur. Moreover, all other preventing factors participate. Growth and toxin production are prevented in a satisfactory way by salting and heating. Living spores can be retrieved in inoculated canned leg of pork, in which the salt concentration is sufficiently high to prevent growth. Spores which are produced in cooked meat are more resistent than those produced in raw meat, but they can be made more susceptible to heat by being suspended for some minutes in a running extract of raw, autolyzed meat. The active substance can be separated from the extract by a fat solvent (Halvorson).

The Initial Flora

The initial flora, or contingent infection of Cl. botulinum in foods due to defilement, should be kept low by strict hygienic measures during all preparation and handling of the foods. Cl. botulinum do not necessarily have to be supplied by soil defilement, etc., since several raw products in themselves can contain microbes or, in the first place, their spores. It has thus been pointed out that such infection is frequent in fish, and H. O. Pedersen has pointed out its existence in the slime on the bottom of the ocean. Furthermore, potato flour, spices, and other raw products which are agricultural products can be infected with spores. Beans have thus contained spores in 31.8% of investigated cases, dried vegetables in 20.6%, root-crops in 16.2%, etc.

It has been found that spores can remain dormant for days, weeks, months, and years before they germinate. The quality of the product is naturally of essential importance in this context.

Synergism and Antagonism

Certain microorganisms have synergetic or symbiotic effects on Cl. botulinum. On the other hand, there are many with antagonistic or antibiotic effects.

The synergetic microorganisms create through their growth favorable environments for Cl. botulinum either because they produce essential substances themselves or because they influence and change the substrate in such a direction.

Thus it has been pointed out (Swartling, Lindgren) that the growth of the butiric acid bacteria can be furthered by other microorganisms, and the growth-furthering effect seems then to be tied to the formation of one or several heat-resistent substances of relatively small molecular size. The effect is sufficiently strong to make growth possible even if strictly anaerobic conditions do not prevail.

It has been observed (Sakaguchi, Tōyama) that Cl. botulinum in pure culture did not produce more than 20 MLD (mouse lethal dose), but when it was allowed to grow in raw culture from the same food, the toxin amount increased 100 times in 6 days. From the raw culture another anaerobic rod was isolated -- it was not toxic to mice and did not produce gas when it grew alone. It furthered the toxin production of the former, but not with regard to Cl. botulinum, type A. The increased toxin production could not be due to any growth-stimulating effect. The produced toxin was neutralized by type E antitoxin. The high toxin production could be reproduced by having washed spores of type E incubated in a sterile culture filtrate of the previously mentioned non-toxic rod at 37° C and pH 5. The responsible substance in the culture filtrates is possibly an enzyme since it did not get dialyzed and destroyed by heat. It is thought that type E cells have a pre-toxic stage in their development and that they require something in order to become toxic.

The investigations suggest a possible relationship between the toxin and the surface of the organism when the maximal toxin production does not occur during the period of strong growth but only after most organisms have been autolyzed (Boroff).

As for the antagonistic effect, there are, on the one hand, microorganisms which directly affect and prevent the growth of Clostridia, and, on the other hand, ones which change the substrate in various ways, for example by producing acids or peroxides, and thereby make growth and toxin formation impossible.

The antagonistic effect of three different lactic acid bacteria towards Cl. botulinum has been studied by Saleh. The results of these investigations suggest that the use of lactic acid bacteria can serve as protection against botulism when prepared frozen foods might possibly be exposed to mal-treatment. The lactic acid bacteria have then to be thoroughly blended into the product, which is thereupon quickly frozen in order to keep the lactic acid bacteria dominant. Such a manner of proceeding seems to be most effective with regard to foodstuffs produced under the best hygienic conditions (Saleh, Ordal)

The preventative effect of the lactic streptococci is stronger than that of lactic bacilli. This is due to the lactic acid produced.

The strong preventative effect in certain cases could not have been effected by neutralization of the acid, but turned out to be due to the production of a peroxide (Hirsch, Mattick).

Lactobacillus acidophilos has proven (Torrey, Kahn) to be able to prevent proteolysis in various proteolytic, spore-producing anaerobes in media favorable to the growth of both.

Natural antibiotics are naturally of great importance. Certain lactic acid-producing bacteria have the ability to produce nisine. Such a nisine-producing rod of Streptococcus lactis has come to be used industrially, especially in the production of certain cheeses; however, one should not forget that the effect of nisine can under practical conditions be uneven, since the rod can for one reason or another lose its nisine-producing ability or be completely destroyed by other microorganisms in the product.

An antibiotic substance, subtilline, produced by Bac. subtilis, has proven to exert a considerable effect. There are two areas within the pH-scale where it is especially active, partly on the acidic side of the neutral point (pH 5.5-6.5) and partly on the alkalic side (pH 8.5-9). In the area between 6.5 and 8.5 the effect is insignificant. In the pH-areas where it is effective, 1 ppm of subtilline checks 10,000 to 100,000 spores, while at pH-values inbetween, the same amount can only check from 1 to 10 spores. Heat has a harmful influence on the effect within the low and high pH-values. At such times, subtilline plus heat only check from 100 to 1000 spores (Krasnow, Jann, Salle). It is thought that subtilline does not affect the spores, but that its effect begins when the germination process has started (Andersen). Other scientists think that Cl. botulinum is noticeably less resistent to heat when it is suspended in foods which contain small amounts of subtilline than in fully subtilline-free products (Le Blanc, Davlin, Stumbo).

Admixture of auromycine to fish filets infected with vegetative cells of Cl. botulinum, type E resulted in a reduction of the number by 40% (Bluhm, Tarr). In other investigations (Kaufmann, Ordal, El-Bisi) Cl. botulinum type B turned out to be insensitive to 9 different antibiotics.

It is further noticed (Lewis, Michener, Stumbo, Titus) that among 67 different antibiotics only 47 in some way prevented growth or lowered the lethal temperature. Subtilline and nisine were active in this respect. There must, therefore, be other chemical substances in some foods, substances which have the ability to increase the thermo-resistance in spores.

An antibiotic which lowers the lethal temperature in regard to spores is helpful in the sterilizing of foods when a low temperature is desirable to prevent too much cooking and to give a wider safety margin. Certain repressing factors can in various ways cooperate and together prevent growth and toxin production, even in concentrations where they alone cannot manage to achieve this.

Environmental Conditions Due to Vacuum-Packing

Vacuum-packed foods are to be regarded as fresh foods, and must therefore constantly be refrigerated. Vacuum-packing is a packing method which protects the product from exterior defilement and which slows down certain chemical processes due primarily to the influence of the air (oxidation, rancidity, dehydration, discoloration). Also from a general bacteriological point of view, the product seems to gain increased stability through correct preparation, handling, and storing.

An absolute condition of the efficaciousness of this method is that only such foods be chosen which are not influenced in an unfavorable direction by the process or which are not changed in one way or another so that the material or the product is affected. First-class raw material, careful, hygienic conditions during the preparation, handling, and storing of the product, and similar conditions during the packing are absolutely necessary. Naturally to this can be added the correct handling, transporting, and storing of the product before it is sold.

When the product is vacuum-packed, the environmental conditions of growth and toxin production of Cl. botulinum seem to be increased or, on the whole, seem to cause increased opportunities for anaerobic bacterial growth. In the investigations which have been made in this field, it has not been possible to point out growth of anaerobes (Alm, Erichsen, Molin); while, on the other hand, the bacterial flora during refrigerated storage has changed in the direction of an almost pure culture of Lactobacillus sp. and Achromobachter sp. Greenberg et alia could not in any case point out toxin production in vacuum-packed fresh meat products before organoleptic, putrid breakdown was clearly noticeable. In many cases there was putrification without toxin production. When vacuum-packed foods are greatly maltreated, these changes should warn the consumer.

According to what Reid states in his unpublished works, toxin of Cl. botulinum could not be pointed out in inoculated "luncheonmeat" vacuum-packed in cellophane pliofilm and stored for 18 days at room temperature. The repressing effect is considered to be due to the salt content, high nitrite content, and sub-optimal storage temperature.

Growth and toxin production of Cl. botulinum can in certain cases occur at such low temperature as 5° C with regard to type E, while in other experiments with type A negative results were obtained at 10° C. Therefore, it is important that refrigeration of vacuum-packed foods always be done at a low temperature.

Kadavy, Dack found that in experimentally-inoculated half-sterile canned foods there was no sign of growth and toxin formation until after 6 months' storage. Living spores could not be found even after 2 years. At a higher water content and pH value, toxin could be found; but not at a lower water content and pH value. The water content was considered to be the limiting factor, and products with a low water content were considered to be bad substrates for Cl. botulinum.

MY OWN EXPERIMENTS

Preparatory Experiments

Before I began the real experiments with vacuum-packed foods, I thoroughly tested the various substrates, partly in order to check on these and partly to gain knowledge about the specific characteristics of the rod utilized and its need for a specific environment. In order to work with inoculated foods on a larger scale, I chose from a purely laboratory-hygienic point of view to work only with a rod of type E, whose spores can easily be extracted from refuse and cultures. The utilized rod of type E came from Div. Laboratories and Research N.Y.S. Dept. Health, and had the designation: 35396.

For liquid substrate and basic medium of agar substrate, I chose infusion of beef heart. In a broth containing this infusion, peptone, common salt, and ground beef (in accordance with Recommended Methods for the Microbiological Examination of Foods 1958) the microbe in a vacuum grows quickly in connection with rapid spore formation. The substrate was kept in 50 g. or 100 g. bottles with screw-caps, which were only half-filled in order to prevent excessive cooking when the vacuum was produced.

The risk of excessive cooking was reduced and the medium even seemed to gain in quality, since all the way from the preparation to the usage it was kept in a vacuum at room temperature and in darkness. In this manner sterility control was also obtained.

In an agar substrate of heart-muscle infusion without admixture, as well as with an admixture of 0.1% Na-thioglycolate, no growth of Cl. botulinum was obtained either when the plates were kept in an anaerobic chamber, or when the 'petri' dishes were vacuum-packed in 'transoten' bags. In some cases there was a teeming growth, but no toxin could be pointed out.

The above mentioned 'transoten' bags are of exactly the same type as those which are used in commercial vacuum-packing. They are made of a combined material whose ingredients are lacquered viscose-film and polyethene with the polyethene layer closest to the product. They are equipped with heat-sealed edges and are closed by heat-sealing. This kind of bag was also used in the experiments which will be related later.

The vacuum was produced by means of an apparatus of the kind used in vacuum-packing, a so-called 'chamber apparatus.' The pumping and sealing of the bag took place in a chamber which was pumped. As with commercial packing, the pumping degree was between 95-98%.

When sediment from an old broth culture, which microscopically was made up almost only of spores, was spread on blood agar /plates/, a typical growth of Cl. botulinum was obtained in a few colonies, the number

of which was not in reasonable proportion to the amount of sediment spread. According to Zeller, Cl. botulinum can only be transferred to blood agar plates when fresh cultures in active growth are used. Any real growth is not possible in solid media, since the composition of these media apparently does not correspond to what Cl. botulinum demands.

Specimens from a fresh (3-day-old) culture were spread on blood agar plates which were partly incubated in an anaerobic chamber and partly packed in vacuum bags in a commercial manner. The incubation was made at 37° C. The growth on the agar plates incubated in the anaerobic chamber was already after three days rich and typical, while the blood agar plates which were vacuum-packed did not show any growth. After 4 days, only one plate out of 16 showed typical colonies of Cl. botulinum corresponding in density to those on the control plates in the anaerobic chamber. Even that plate showed an additional, unspecific growth. The isolated unspecific rod (staphylococcus) allows growth of Cl. botulinum on blood agar, according to Fortner.

After the blood plates were kept vacuum-packed at room-temperature for 1 month, most of them (11) showed typical growth, but at the same time more or less rich unspecific growth (see above).

When admixing 0.1 or 1% cysteine, a crystalline precipitation was obtained in bowls kept both in an anaerobic clock and in vacuum-packs. It was obtained at the places where the culture was spread, and the amount corresponded to the material spread. The precipitation was greatest on plates with 1% cysteine. After 10 days a rich growth, most often of lytic Clostridia without significant spore formation, could be noticed in this precipitation. Within areas where much culture had been spread, free spores were found (from the original culture?). Even after 10 days and up to a month after the culture was spread, a thin surface growth with thread-like, meandering offshoots could be noticed between the precipitated crystals.

These were easiest to notice in the concentration with a ½% cysteine, where the distance between the precipitated crystals was greater. The growth was identical on plates from the anaerobic chamber and the vacuum bags. When it was transferred to a broth, toxin was produced.

Growth of Cl. botulinum can thus occur in the reduced oxygen situation which vacuum-packing represents, but only if other conditions are especially favorable, for example, if there exists some other reducing micro-flora and easily autolysable material (blood).

If the substrate, as in practical life, is a food article with varying composition, the question of the influence of furthering and repressing factors on growth and toxin production naturally becomes complicated. It is therefore necessary to gain experience in the influence

of different, widely-used foods on the growth and toxin production of Cl. boutlinum under circumstances which prevail at the time of vacuum-packing, i.e., to gain experience of the botulinogenity of the various products.

Experimental inoculation of various foods which will be vacuum-packed and stored under conditions favorable to Cl. botulinum, as well as consequent investigation regarding toxin possibly produced must be performed in great series.

In an initial experimental series, the inoculation was performed by spreading a spore suspension in the form of a little loop on a slice of the product. This spore suspension had been obtained by centrifugalizing a 10-day-old broth culture and by washing the sediment once with a physiological common salt solution. A corresponding slice was put on top, and both were immersed in a 'transoten' bag, which was pumped in commercial manner. In one bag series, the vacuum pressure was eliminated immediately. The incubation took place at 30° C. The extract was prepared after being stored for various periods of time by admixing the least possible amount of liquid.

	7 days				15 days				1 month				4½ months		
	V		Ö		V		Ö		V		Ö		V		
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	3
Veal roll	0	0	+	0	-	-	-	-	+	0	-	-	-	-	-
Cooked meat sausage (medvurst)	0	0	+	0	-	-	+	0	(+)	0	-	-	+	0	+
Domestic meat sausage (hushaellsmedvurst)	0	0	(+)	0	-	-	-	-	0	0	-	-	0	0	+
Cooked butts roast (grishals)	0	0	(+)	0	-	-	-	-	0	0	-	-	-	-	-
Sandwich leg of pork	-	-	-	-	0	0	0	0	0	0	-	-	0	0	+
Sauna-smoked beef	-	-	-	-	0	0	+	0	0	+?	-	-	-	-	-
Special pork	0	0	+	0	-	-	-	-	0	0	-	-	0	0	+
Pork roll	0	0	+	0	-	-	+	+?	+	+?	-	-	-	-	-
Hamburger meat	-	-	-	-	0	+	+	+	+	+	-	-	-	-	-

V : vacuum-pack
O : open bag
Column 1 : organoleptic changes
Column 2 : appearance of toxin
Column 3 : production of toxin in broth culture from the product

\+ : presence of toxin and organoleptic changes
0 : presence of toxin not indicated
\- : investigation not finished

These experiments, which were of a preparatory character, produced the following results:

The different products do not seem especially suited to growth and toxin production of Cl. botulinum. It could be noticed, however, that hamburger meat and pork roll had a toxic effect in mice. Mice inoculated with extract from hamburger-meat showed symptoms typical of botulism, while mice inoculated with extract of pork roll died under uncontrolled conditions. The toxin was thermolabile. Specific anti-serum was not available at the time.

The toxin production was most often tied together with organoleptic changes. The toxin was pointed out simultaneously in vacuum-closed and open bags, and on one occasion (pork roll) in an open but not in a vacuum-closed bag. Thus the vacuum as such did not seem to be of decisive importance.

The microbic flora of the product can be of importance under seemingly aerobic conditions and allows growth and toxin production through symbiosis.

The spores keep dormant over a long period of time. These results were gained for the following experiments:

1. The assortment of products should be broadened to comprise fish products, as well as remaining meat products, which can be subject of vacuum-packing.

2. The inoculation should be standardized and based on the age of the culture (10 days) and should be applied in the form of suspension (c. 10^7 spores/ml).

3. The vacuum should be eliminated in the control bags by air immersion.

4. Incubation of inoculated material occurs (unless nothing else is indicated) at 24-25° C.

5. The preparation of the extract should be standardized to a 10% suspension of the product in a physiological common salt solution. This concentration is chosen in order that the material used shall not disturb the result of the investigation, and also so that the experiment shall resemble natural conditions. This is because it has turned out to be very difficult to rid the spores fully of toxin by washing. Small amounts of toxin do not come into account in practical conditions. The smallest dose which can be observed when a 10% suspension is used is (at injection of 0.5 ml) 20 MLD per g. of product. If a mouse weighs 20 g. it corresponds to a living mass of 40 kilos in connection with a dose of the product amounting to 100 g.

6. In order to ensure that the infection really occurs, immersion of the material should be performed in every single case, suitably from the sediment after the centrifugalizing of the suspension is finished. The broth should be checked for presence of toxin after from 4 to 6 days of incubation in a vacuum at 30° C.

7. The pH should be controlled in the suspension in each single case.

8. Possible aerobic culture should be made from the suspension.

9. Other necessary investigations should be made to clarify the specific ability of certain foods to assist in growth and toxin production, as well as the (specific) repressing factors which may possibly constitute obstacles for this.

10. For the diagnosis, inoculated mice should show characteristic symptoms and die within 24 hours, but should be observed for three days and nights. After 3 days mice with a-typical symptoms and mors should be regarded as unspecific reactions. If death occurs on the second or third day, the diagnosis cannot be determined unless further experiments are made, possibly in connection with a neutralization test.

Experiments With Fresh Herring

Fresh herring is very botulinogenic in respect to type E. It has therefore proven to be a perfect medium both for control of inoculations in connection with experimentally infected, vacuum-packed foods, and as a medium for cultures in connection with investigations of defilement sources.

Fresh herring is gutted, boned, and rinsed; afterwards, it is drained or wiped off with a filter paper. Before inoculation a little piece is cut from each herring for control of naturally occurring infection. The inoculated and the uninoculated parts are packed in separate 'transoten' bags which are kept in a vacuum for from 4 to 6 days, when a toxin-check is made. (The uninoculated part naturally does not need to be checked except in positive cases.) In practice, it has proven possible in connection with naturally occurring outbreaks of type E botulism to produce pure Cl. botulinum from a 10% suspension of herring thus inoculated, either directly from the suspension or indirectly from a broth. In this case, it was very difficult to obtain toxic sub-cultures in the usual manner.

Experiments With Inoculated Fresh Herring

	Period of storage days 22°C	10% suspension V	0	(MLD/g)
Fresh	4	+ (800)	+ (800)	
Cooked	4	+ (200)	+ (200)	
Fresh	7	+ (1000)	-	
Cooked	7	+ (1000)	-	
Ground Herring	4	+ (500)	+ (500)	
Iron Serum	4	+	+	
Extract	4	+	+	
Iron Serum Sterilized	4	+	-	not titrated
Iron Serum Filtred	4	+	+	

As can be seen, the vacuum does not seem to be of decisive importance, even when the growth and toxin formation occur under seemingly strict anaerobic conditions. Nor does it seem to make any difference whether or not the herring is cooked, or whether the egg white has been precipitated by means of colloidal iron hydroxide or by adjusting the pH value to the isoelectric point and heating to 70° C. Toxin production occurs whether serum or extract is used in raw, filtered, or sterilized condition.

Experimentally Infected Salt Herring (Fladen Herring) in Vacuum-Pack

Period of storage, 25° C, in weeks	Toxin in 10% suspension		Toxin production in broth in culture from suspension	
	V	0	V	0
1	0	0	-	-
2	0	0	+	+
5	0	-	+	-
20	0	-	+	-
2½ weeks at 5° C plus 3 weeks at 30° C	0	-	+	-

The salt content is sufficiently high to prevent growth and toxin production completely. Storage at 25° C does not cause any organoleptic changes in the herring. At 30° C the product dissolves and forms a gruel-like mass, which has an aromatic, fresh smell.

Experimentally Infected Soaked Salt Herring in Vacuum-pack

The herring was soaked in running water for about 18 to 24 hours. The filets were inoculated and vacuum-packed in double and single layers, respectively.

Period of storage, 25° C, in days	Toxin in a 10% suspension				Toxin production in broth culture from suspension			
	Double		Single		Double		Single	
	V	O	V	O	V	O	V	O
5	+	-	+	-	-	-	-	-
9	-	+	-	0	-	-	-	-
12	-	-	-	+	-	-	-	+
14	-	-	-	+	-	-	-	+

The toxin production can be indicated whether the herring was packed in a vacuum or not, and in single as well as in double layers. The salting did not give the product any enduring changes with growth-repressing effect.

Experimentally Infected "Seelachs" (Colored Coalfish) in Vacuum-Pack

Period of storage, 25° C, in weeks	Toxin in 10% suspension		Toxin production in broth culture from suspension	
	V	O	V	O
1	0	0	-	-
2	0	0	+	+
5	0	0	+	+
2½ weeks, 5° C plus 3 weeks, 30° C	0	0	+	+

The salt content is sufficiently high to prevent growth and toxin production completely. When stored over a longer period of time at high temperature, the color disappears gradually from the product. In the end it is completely colorless.

Experimentally Infected Filets of Norway Haddock (Sebastes Marinus, in Norwegian "uer") in Vacuum-pack

A 300-ml, 9-day-old toxic broth culture was centrifuged. For the control of the infected rod we made neutralization tests, which gave positive and unambiguous results. The sediment was washed and finally immersed in 300 ml of physiological common salt solution. From this suspension we took 30 and 3 ml., respectively, for new suspensions, each with a total volume of 300 ml, so that a series with the concentrations of 1, 1:10, and 1:100 was obtained.

The filets were dipped in respective suspensions, were drained on a filtre paper, and were then packed two in each vacuum-bag. In half the bags the vacuum pressure was eliminated by air immersion with the aid of a tube. All packs were incubated at 25° C.

After 48 hours there was obvious gas development in all packs. After 72 hours it was so strong that the storage had to be interrupted. The product had a strong putrid smell, which almost made further work impossible.

The 10% suspensions were made in a series, consisting of three packs (various doses of infection) which were kept in a vacuum and three packs which were in open bags. The suspensions were centrifugalized at 3,200 revolutions for 1 hour. 0.5 ml of the centrifugalate of each was inoculated into mice. All mice showed clear and typical symptoms 1½ hours after the injection.

Period of storage, 25° C, in days	V			0		
	1	1:10	1:100	1	1:10	1:100
3	+ (2:05)	+ (3:15)	+ (2)	+ (1:40)	+ (3)	+ (4)

( ) : numbers of hours after the injection.

Closer titration of the strength of the toxin has not been made. It seems, however, that the toxin was very strong (estimated to > 2000 MLD/g). The product can be regarded as extremely botulinogenic.

Commercial vacuum-packing before freezing such a botulinogenic product would seem to be a hygienic venture; however, such is probably not the case, since growth conditions are fully as good under what are apparently not strictly anaerobic conditions. The strong gas development and the putrid smell are alarming symptoms for the consumer. Moreover, weak toxin doses possibly present could be destroyed during preparation and cooking.

Toxin Production in Inoculated Ground Meats

The various ground meats were contained in 100 g. bottles, of which half were incubated in a vacuum and half in an open can.

4 days, 25° C	Toxin in 10%-suspension	
	V	0
Calf heart	+	+
Herring	+	+
Cod	+	-
Shrimp	0	0

Toxin production takes place in botulinogenic products also under conditions seemingly not strictly anaerobic.

Experiments to Discover Whether Toxin Production of Cl. botulinum Takes Place in Artificially-Infected Cooked Shrimp, With Special Regard to Vacuum-Packs (Vacuum-Packed Before Being Deep-Freezed)

Experiment I. Infected Vacuum-Packed Shrimp

Period of storage, 25° C, in days	Toxin in 10%-suspension		Toxin-production in broth culture from the suspension	
	V	O	V	O
2	0	0	+	+
3	0	0	0	0
4	0	0	+	+
5	0	0	+	0
6	0	0	+	+
10	0	-	-	-
12	0	-	+	-
14	0	-	+	-
32	0	-	+	-
32	0	-	+	-
32	0	-	+	-
32	0	-	+	-

Experiment II. Infected Shrimp, Stored in an Anaerobic Chamber at 30° C

Period of storage in days	Toxin in 10%-suspension	Toxin production in broth culture from the suspension
6	0	+
12	0	+
19	0	+
25	0	+
32	0	+

Experiment III. Ground meat of Infected Shrimp, Stored in Bottles at 22° C

4 days		8 days		12 days	
V	0	V	0	V	0
+(4)	+(4)	-	+(3)	-	+(5)
			3 mice		3 mice

( ) : number of 24-hour periods after the injection

The deaths occurred in a manner not usual for botulism. No breathing difficulty or costal breathing. Lethargy, closed eyes, no water consumption, desiccation, quiet death.

Neutralization experiments with botuline E sera give negative test for botulinus toxin.

Experiment IV. Preparation of Extract (Iron Serum) of Shrimp

pH 6.5. The extract infected with Cl. botulinum.
Incubation at 22° C.

4 days		8 days	
V	0	V	0
+(6)	0	+(6)	+

( ) : number of 24-hour periods after the injection.

The deaths occurred as under III.
Neutralization experiments not made.

Experiment V. Investigations in Dialysate and Dialyzed Ground Meat

Ground meat of shrimp diluted with water (thin gruel consistency) was dialyzed for 2 x 24 hours against distilled water. The dialysate was concentrated to two-thirds of its original volume of shrimp. The pH was 6.3. It was infected with Cl. botulinum and kept in bottles, the dialysate in a vacuum and the ground meat in a closed bottle, at 25° C for 10 days.

Dialysate MLD/ml			Dialyzed ground meat MLD/g	
0	or	2	more than 1000	
2	+	0	20	+ (3 hours)
20	0	0	200	+ (5 hours)
200	0	0	500	+ (23 hours)
			1,000	+ (10 hours)

Experiment VI. Infected Vacuum-packed Shrimp. 12 days, 25° C

Neutralization tests of toxin of culture used for infection in the following experiments have been made with one single result.

10%-suspension		
Toxin in 0.5 ml of the centrifugate ( ) : number of 24-hour periods after the injection	Toxin in a 0.5 ml filtrate (sterile)	Toxin production in broth culture from the suspension ( ) : number of hours after the injection
+ (4)	0	+ (4:30)
+ (6)	0	+ (<18)
+ (6)	0	+ (5)
+ (7)	0	+ (5)
+ (5)	0	+ (<18)
0	-	+ (<18)
0	-	+ (<18)
+	0	+ (<18)
0	-	+ (48)
0	-	+ (<18)

Experiment VII. Infected Shrimp Stored in an Anaerobic Chamber for 12 Days at 25° C

10%-suspension			
Toxin in 0.5 ml of the centrifugate ( ) : number of 24-hour periods after the injection	Toxin in 0.5 ml of filtrate (sterile)		Toxin production in broth culture from the suspension ( ) : number of hours after the injection
	1	2	
+	+ (8 days)[x]	0	+ (2)
0	-		+ (2:50)
0	-		+ (2:50)
+	0		+ (2:30)
0	-		+ (4:30)
0	-		+ (<4)
0	-		+ (4)
+	0		+ (2:05)

x) Neutralization test negative. Not botulism.

Experiment VIII. Infected Shrimp, Ground into Meat (Without Admixture of Water) and Stored in an Anaerobic Chamber at 25° C. 12 Days

A. Toxin in 0.5 ml of centrifugate ( ) : number of 24-hour periods after injection	Toxin in 0.5 ml of filtrate (sterile)	Toxin production in broth culture from the suspension ( ) : number of hours after injection
+ (3)	0	+ (2:50)
+ (4)	0	+ (2:05)
+ (2)	0	+ (<4)
+	0	+ (2:40)
+ (5)	0	+ (2:50)
+ (3)	0	+ (<4)
+ (2)	0	+ (4:30)
B. With admixture of water.		
0	-	+ (<4)

The experiments seem to suggest that shrimp under normal conditions is quite resistent to the production of toxin after growth of Cl. botulinum. It seems practically impossible to bring about such toxin production, either in partial vacuum or under more complete anaerobic conditions. It is not yet known what causes this resistance mechanism. As well as the importance that the way the product breaks down under normal circumstances can have in this respect, there are also more or less complicated biochemical and physical conditions. The water content in shrimp is between 75-80%, but this is no expression of the equilibrium relative humidity of the product or its content of active water, upon which the solution of essential nutrients is dependent. Experiments with dialyzed ground meats, which turned out to be strongly botulinogenic, suggest that the repressing effect is to be found among such factors. It is less probable that the repression is due to solution of specific substances.

The changed condition of the product through cooking is naturally of importance for this resistance from a physical-chemical point of view. It should, therefore, be of special interest to make similar investigations on raw shrimp, which, however, has not yet been possible.

Vacuum-packing before the freezing of shrimp is of no importance from a hygienic point of view with regard to the growth and toxin production of Cl. bitulinum. This is naturally assuming that the normal constitution of the product is not tampered with, and that handling, treatment, preparation, and storage take place in a normal hygienic manner.

Experiments with Inoculated Ground Meat of Cooked Crab

Cooked crab was ground in a meat-grinder, and the released brine was immersed into the ground meat. The ground meat was contained in 100 g bottles, each of which contained 50 g., and inoculated with 1 ml from a washed spore suspension containing 10^7 spores. They were incubated in a vacuum at 25° C.

Period of storage in days	Toxin in 10% suspension	Toxin production in broth culture from the suspension
3	0 pH 6.7	+ (2)
6	0 pH 6.9	+ (2½)
9	0 pH 6.9	+ (2)
12	0 pH 7.2	+ (2½)
16	0 pH 7.6	+

( ) : number of hours until death occurred after injection.

There seems to be the same resistence to infection or toxin production as in shrimp. From the sixth day, the product was dissolving into a thin gruel, exuding a putrid smell.

Experiments With Infested Dialysate and Infected Ground Meat of Various Kinds

The ground meats were put in dialysis tyres, amounting to between 100-150 g. in each. Occasionally water was admixed so that a thin gruel consistency was obtained. They were dialyzed with distilled water for 2 x 24 hours, during which time the ground meat was repeatedly stirred, and at a temperature not exceeding 5° C. The dialysate obtained was in each case concentrated to two-thirds the amount of the original material. Dialysate and ground meat were inoculated with spore suspension. Incubation was in a vacuum at 25° C. for 10 days.

	Dialysate, filtrated MLD/ml		pH in dialysate	Dialysed ground meat. 10%-suspension		pH in suspension
Beef	2	0	5.2	200	0	6.8
	20	0		500	0	
	200	0		1000	0	
Pork Heart	2	+	6.6	200	+	7.4
	20	+		500	+	
	200	+		1000	+	
Herring	2	+	6.5	200	+	6.3
	20	+		500	+	
	200	0		1000	+	
Cod	2	+	7.0	200	+	6.7
	20	+		500	+	
	200	0		1000	0	
Deep-freezed peas	2	0	4.6	200	0	4.2
	20	0		500	0	
	200	0		1000		
Deep-freezed spinach	2	+	6.2	200	0	4.8
	20	0		500	0	
	200	0		1000	0	
Shrimp + water (thin gruel consistency) cooked	2	+? 0	6.3	200	+ (3)	6.3
	20	0		500	+ (5)	
	200	0		1000	+ (10)	( ) = number of hours after injection
Crab cooked	2	0	7.5	200	0	7.6
	20	0		500	0	
	200	0		1000	0	
Skim milk	2	0	5.8		-	
	20	0			-	
	200	0			-	
Separator sludge	2	0	5.2		-	
	20	0			-	
	200	0			-	

Beef, deep-freezed peas, deep-freezed spinach, cooked crab, skim milk, and separator sludge cause no growth or toxin production of Cl. botulinum, type E, either in dialysate or ground meat; while shrimp, as is mentioned earlier, showed toxin production in water-diluted ground meat but not in dialyzate. The experiment showed to what extent these products could serve as media for growth and toxin production (botulinogeneity).

Experimentally-Infected Smoked Fish Products in Vacuum-Pack

Period of storage 22-25° C, in weeks	Toxin in 10%-suspension	Toxin production in broth culture from suspension
	Smoked Eel	
2	0 pH 6.8	0
3 (putrid)	0 pH 6.9	+
4 "	0 pH 6.6	0
5 "	0 pH 7.2	+
	Smoked Mackerel	
2	0 pH 6.3	+
4	0 pH 6.6	+
12 (putrid)	0 pH 6.6	+
	Smoked Herring	
3	0 pH 6.6	0
4	0 pH 6.2	0
5	0 pH 6.0	+
12	0 pH 6.0	+

Smoking and other treatment have in these products changed the substance in such a manner that growth and toxin production of Cl. botulinum is made impossible.

Meat Products Inoculated with Spore Suspension and Incubated in an Anaerobic Chamber at 30° C

	Period of storage, weeks	Toxin in 10%-suspension		Toxin production in broth culture from suspension
Collared head	1	0	pH 5.6	+
	2	0	pH 6.3	+
Liver paste	1	0	pH 5.4	+
	2	0	pH 5.1	+
Veal brawn	1	0	pH 5.2	+
Cooked Boston butts	1	0	pH 5.5	+
	6	0	-	+

(continued)

(continued)

	Period of storage, weeks	Toxin in 10%-suspension		Toxin production in broth culture from suspension
Cooked beef	1	0	pH 5.8	+
	6	0	pH 6.1	+
Ham sausage	1	0	pH 5.2	+
	6	0	pH 5.9	+
Cooked leg of pork	1	0	pH 5.7	+
	6	0	pH 6.8	+
Cooked meat sausage (pork)	1	0	pH 5.2	+
	6	0	pH 5.8	+

Even under strictly anaerobic conditions and at a favorable temperature, it does not seem possible to obtain toxin production within a reasonable time in the above inoculated products.

Experimentally Infected Meat Products in Vacuum-pack

Incubation at 22-24° C

	Period of storage, weeks	Toxin in 10%-suspension		Toxin production in broth culture from suspension
Liver paste	1	0	pH 5.5	+
	2	0	pH 5.8	+
	8	0	pH 4.2	+
	10	0	pH 4.8	+
	10	0	pH 4.2	+
	13	0	pH 4.0	+
Liver paste + Serratia	1	0	pH 6.0	+
	2	0	pH 6.4	+
	10	0	pH 4.8	+
Liver paste + Koli	1	0	pH 5.5	+
	2	0	pH 5.2	+
	10	0	pH 4.5	+
Liver paste + Serratia extract	1	0	pH 6.0	+
Liver paste + Koli extract	1	0	pH 6.0	+
Hamburger meat	3½	0	pH 5.2	+
	8	0	pH 4.8	+
	10	0	pH 5.5	+
	13	0	pH 4.8	+
Pork roll	3½	0	pH 5.5	+
	10	0	pH 5.5	+

(continued)

	Period of storage, weeks	Toxin in 10%-suspension		Toxin production in broth culture from suspension
Pressed veal	3½	0	pH 4.8	+
	10	0	pH 4.8	+
Sausage	1	0	pH 4.8	+
	2	0	pH 5.2	+
	4	0	pH 4.7	+
	5	0	pH 4.5	+
	10	0	pH 4.5	+
Brawn	10 (putrid)	0	pH 6.6	+
Wiener sausage	8	0	pH 4.0	+
Pork	8	0	pH 5.8	+
	13	0	pH 5.6	+
Salt beef	8	0	pH 6.1	+
	13	0	pH 5.8	+
Salami	8	0	pH 4.8	+
	13	0	pH 4.8	+
Bacon	8	0	pH 5.8	+
	13	0	pH 5.6	+
"Berliner" cooked meat sausage	8	0	pH 4.2	+
"Goinge" sausage	8	0	pH 4.0	+
Smoked leg of pork	8	0	pH 5.2	+
	13	0	pH 5.5	+
"Bayonne" leg of pork	8	0	pH 6.6	+
	13	0	pH 6.6	+
"Bier" leg of pork	8	0	pH 4.5	+
	13	0	pH 4.5	+
Domestic meat sausage	8	0	pH 4.2	+
Cooked leg of pork	8	0	pH 6.0	+
	13	0	pH 6.4	+

CONCLUSION

It is a fact that on no occasion have we been able to point out toxin production in the product in our investigations in spite of favorable conditions of temperature and time. Such long storage would probably never occur in practice, even at low temperature. It can be concluded that these products do not constitute favorable media for growth and toxin production.

The reasons can be many and varying. The pH value in the product is changed in many cases in such a manner that toxin production during storage is made impossible.

The water content is in most cases below 50%, and the equilibrium relative humidity is probably usually very low, which to a great degree represses growth and toxin production. The conditions of growth and toxin production are not attained even by admi ture of such micro-flora, which can be conducive to reduction of the red-oxpotential. Neither is vacuum-packing decisive in spite of the fact that it tributes to the maintenance of a low red-oxpotential.

The NaCl, $NaNO_3$, and $NaNO_2$ contents as well as other antagonistic substances are in most cases unknown but can naturally function as repressing factors like cooking, smoking, and other treatment.

The possibilities of growth and toxin production in commercially vacuum-packed foods cannot be traced merely to a single favorable factor, such as, for example, the production of a vacuum, since the counteracting factors are overwhelming. It can therefore be established that these possibilities are fully dependent on the kind and nature of the product itself and to the product's degree of botulinogeneity. In this respect, the inclination to autolysis is a primary determinant ("primary botulinogeneity.")

In the second place, we have the remaining microbial flora, mainly proteolytic, which can contribute to the breakdown of the product ("secondary botulinogeneity"). When it comes to botulinogenic substances or products, the risks of growth and toxin production seem to be equally great whether or not the product is vacuum-packed. Production of a vacuum can in such cases contribute to creating alarming cautionary symptoms noticeable to the consumer.

Summary

In a general survey, based on the literature in the field, the influence of various environmental factors on the growth and toxin production has been explained. The chemical composition, the pH value, the red-oxpotential, the physical situation, the various changes through preparation, the initial micro-flora, the synergism and antagonism, etc., of the product are factors which regulate and limit the possibilities here.

When it comes to vacuum-packed foods and the possibility of growth and toxin production due to the apparently anaerobic conditions, it turns out that bacuum in itself is not of essential importance but that the possibilities of growth and toxin production are fully dependent upon the kind and nature of the substance itself. Its disposition to autolysis is decisive for its botulinogenity.

CLOSTRIDIUM BOTULINUM - A BIBLIOGRAPHY

by
Alf Erichsen and Inger Erichsen
Swedish Institute for Preservative Research

The bibliography is summary. It is arranged (a) alphabetically by the first author, (b) chronologically, and (c) alphabetically by the following authors. Anonymous works are listed under the name of the magazine or the publishing institution.

Abrams, A., Kegeles, G. & Hottle, G.A. 1946. The purification of toxin from Cl.bot. type A.
J. Biol. Chem., 164, 63-79.

Abrosimow, V.N. 1956. Mechanism of action of botul. toxin on respiration.
(Exp. Study.) Arch. pat., 18, 86-92.

Aitken, R.S., Barling, B. & Miles, A.A. 1936. A case of botulism.
Lanc. 780-784.

Akkermans, J.P.W.M. 1958. Botulism of minks.
Tijdschr.v.Diergeneesk, 83, 487-492.

Allen, R.W. & Ecklund, A.W. 1932. Botulism in North Dakota.
J.A.M.A., 99, 557-559.

Ambache, N. 1949. The peripheral action of Cl.bot. toxin.
J. Physiol., 108, 127-141.

Ambache, N. 1951. Unmasking after cholinergic paralysis by bot. toxin, of a reversed action of nicotine on the mammalian intestine revealing the probable presence of local inhibitory ganglion cells in the enteric plexuses.
Brit. J. Pharmacol., 6, 51-67.

Ambache, N. 1951. A further survey of the action of Cl.bot. toxin upon different types of autonomic nerve fibre.
J. Physiol., 113, 1-17.

Ambache, N. 1952. Effect of bot. toxin upon the superior clinical ganglion.
J. Physiol., 116, 9-

Ambache, N. & Lessin, A.W. 1955. Classification of intestinomotor drugs by means of type D bot. toxin.
J. Physiol., 127, 449-478.

Andersen, A.A. 1951. A rapid plate method of counting spores of Cl.bot.
J. Bacteriol., 62, 425-432.

Andersen, A.A. 1952. Effect of subtilin on spores of Cl. bot.
J. Bacteriol., 64, 145-149.

Andersen, A.A., Michener, H.D. & Elcott, H.S. 1953. Effect of some antibiotics on Cl.bot.
Antibiotics & Chemotherapy, 3, 521-526.

Ando, Yoshiaki & Inoue, Katsuhiro, 1957. Studies on growth and toxin production of Cl.bot. type E in fish-products.
I. On the growth in relation to the oxydation-reduction potential in the fish flesh.
Bull. Jap. Soc. Sci. Fisheries, 23, 458-462.

Appell, R.N., Hartsough, G.R., MacCoy, E.F. & Brandly, C.A. 1956. Active and passive protection of mink against type C botulism with type C toxoid and polyvalent antitoxin.
Am. Vet. Med. Ass. J., 128, 556-558.

Appleton, G.S. & White, P.G. 1957. Cl.bot. type C. I. Studies on laboratory variables affecting toxin production and toxoid evaluation.
Am. J. Vet. Res., 18, 942-946.

Appleton, G.S. & White, P.G. 1959. Field evaluation of Cl.bot. type C toxoids in mink.
Am. J. Vet. Res., 20, 166-169.

Appleton, G.S. & White, P.G. 1959. Laboratory evaluation of a trivalent toxoid for botulism.
Am. J. Vet. Res., 20, 170-172.

D'Arca, S. & D'Arca-Simonetti, A. 1955. Detoxifying power of free active chlorine on botulinus toxin type A.
Nuovi ann. igiene e microbiol., 6, 258-266. Ibid. 6, 355-360.

Australia, Dept. of Health. 1953. Diseases of domestic animals in Australia. Part 5, vol. 1: Bacterial diseases, Sydney (Service Publ. (Div. of Vet. Hygiene) No. 9).

Avery, R.J. & Dolman, C.E. 1959. A nat. outbreak of Cl.bot. type C.
Can. J. Comp. Med., 23, 203-209.

Bačun, M. 1958. Botulism; 4 case reports. Liječ vjes (Zagreb), 80, 310-

Bachmann, F.M. 1924. Growth of Cl.bot. in fermented vegetables.
J. Infect. Diseases, 34, 129-131.

Bachmann, F.M. & Haynes, D. 1924. Prevalence of toxin-producing anaerobes in Wisconsin.
J. Infect. Diseases, 34, 132-136.

Bang-Jensen, B. & Hahnemann, F. 1959 Botulisme.To udbrud med stor forlöbsmaessig lighed. [Botulism. Two Out-Breaks of Great Similarity]
Ugeskrift for Läger, 121, 1363-1368.

Bard, R.C. 1950. Mechanism of action of clostridial toxins.
Proc. Indiana Acad. Sci., 60, 67-72.

Barnes, E.M. 1956-57. Antibiotics for preservation of perishable food.
Proc. Inst. Refrig., 53, 85-95.

Barron, A.L. & Reed, G.B. 1954. Cl.bot. type E toxin and toxoid.
Can. J. Microbiol., 1, 108-117.

Beck, J. 1940. Botulism in a cat.
Jahresberichte für Vet. Med., 67, 303.

Behre, A. 1955. Kritische Betrachtungen über den Nachweis bakterieller Lebensmittelvergiftungen, insbesondere von Fisch Botulismus.
Dtsche Lebensmitt. Rdsch., 51, 83-84, 117-120, 139-144 & 163-168.

Bekker, J.H. 1955. Botulism in Netherland. Conserva, 3, 198-201.

Bell, J.F., Sciple, G.W. & Hubert, A.A. 1955. A microenvironment concept of the epizoology of avian botulism.
J. Wildlife Management, 19, 352-357.

Bell, J.F., Farrell, R.K. & Gorham, J.R. 1957. A polyvalent toxoid for botulism in mink.
Amer. J. Vet. Res., 18, 167-170.

Benard, H., Rambert, P. & Pestel, M. 1943. Botulism from preserved goose.
Bull. Hyg., 18, 758.

Bengtson, I.A. 1921. Direct inoculation test for B. botul. toxin.
Publ. Health Rep., 36, 1665-1671.

Bengtson, I.A. 1922. Preliminary note on a toxin producing anaerobe isolated from larvae Lucilia caesar.
Publ. Health Rep., 37, 164-170.

Bengtson, I.A. 1923. Toxin producing anaerobe isolated from fly larvae.
Publ. Health Rep., 38, 340-344.

Bengtson, I.A. 1924. Studies on organisms concerned as causative factors in botulism.
Hyg. Lab. Bull. (Wash.), 136, 1-96.

Benjamin, M.I.W., Wheater, D.M. & Shepherd, P.A. 1956. Inhibition and stimulation of growth and gas production by clostridia.
J. Appl. Bact., 19, 159-165.

Bennetts, H.W. & Hall, H.T B. 1938. Botulism of sheep and cattle in Western Australia: its cause and its prevention by immunization.
Australian Vet. J., 14, 105-118.

Bergman, R.K. & Lindblad, Y. 1934. En familjeepidemi av botulism. (5 observerade fall). (A Family Epidemic of Botulism (5 observed cases)]
Sv. Läkaresällskapets forhandl., 39-48.

Borgman, R.K. 1934. Botulism and its occurance in Scandinavia.
Nord. Med. Ti., 8, 949-954.

Bergman, R.K., Insulander, S. & Lindblad, Y. 1935. A serologically verified epidemic of botulism in Stockholm. Involving 5 members of a family.
Acta Med. Scand., 84, 496-516.

Bever, J.S. & Halvorson, H.O. 1947-48. The possibilities of the growth of Cl. bot. in canned bread.
Hormel Inst. Univ. Minnesota, 53-58.

Bianchi, E. 1958. Botulism in domestic animals. (In Italian.)
Clin. Vet., 75, 71-83. Ibid. 102-118.

Birge, E.A. & Juday, C. 1922. The inland lakes of Wisconsin. The plankton.
I. Its quantity and chemical composition.
Wisconsin Geological and Natural History Survey, Bull. No. 64, Scientific Series No. 13.

Bishop, O. & Bronfenbrenner, J. 1936.
J. Am. J. Physiol., 117, 393-404.

Bjerke, O. et al. 1959. Botulism from trout.
Tiskr. Norsk Laegefor., 79, 249-251.

Blair, E.B. 1950. Observations on bacterial spore formation in synthetic media. (Ref.) Texas Rep. Biol.& Med., 8, 361.

Bluhm, H.M. & Tarr, H.L.A. 1956. Effect of chlortetracycline treatment of fish on development of certain food-poisoning bacteria.
Fish. Res. Bd Can., Progr. Rep.Pacific Coast St., 106, 8-10.

Bodenmüller. 1834. Über Wurstvergiftung.
Wurt. Cor.-Bl., 30.

Bonventre, P.F. et al. 1959. Physiology of toxin production by Cl.bot. types A and B. II. Effect of carbohydrate source on growth, autolysis and toxin production. III. Effect of pH and temperature during incubation on growth, autolysis and toxin production.
Appl. Microbiol., 7, 372-377.

Boor, A.K., Tresselt, H.B. & Schantz, E.J. 1955. Effects of salts and colloids on potency of botulinum toxin.
Proc. Soc. Exptl. Biol. Med., 89, 270-272.

Boroff, D.A., Raynaud, M. & Prévot, A.R. 1952. Studies of toxin of Cl.bot. type D. J. Immunol., 68, 503-511.

Boroff, D.A. & Caboon, M.D. 1954. Studies of toxins of Cl.bot. II. Immunization of rabbits with an atoxic variant of Cl. bot. type C.
J. Bact., 67, 198-201.

Boroff, D.A. 1955. Study of toxins of Cl. bot. III. Relation of autolysis to toxin production.
J. Bact., 70, 363-367.

Boroff, D.A. & Reilly, J.R. 1958. Active immunization of pheasants and ducks against Cl.bot. type C intoxication.
Soc. Amer. Bact. Proc., 58, 78-79.

Boroff, D.A. et al. 1958. Flourescence of toxin of Cl.bot. and its relation to toxicity.
Nature, 181, 751-752.

Boroff, D.A. & Reilly, J.R. 1959. Studies of the toxin of Cl.bot. V. Prophylactic immunization of pheasants and ducks against avian botulism.
J. Bact., 77, 142-146.

Botija, C.S. 1954. Lo botulisme des équidés en Espagne (epizootologie clinique, traitement et prévention).
Off. Int. des Epiz. B., 42, 759-764.

Bouisset, L. et al. 1956. Untersuchungen über den Stoffwechsel von Cl.bot. A.
Compt. rend. Soc. Biol., 150, 2007-2010.

Bouisset, L. et al. 1957. Aminosäuren nachgewiesen in einer Kultur von Cl. bot. A in VF glukose Nährlösung.
Compt. rend. Soc. Biol., 151, 176-

Bouisset, L. et al. 1957. Wachstum und Toxin-Bildung bei Cl.bot. A.
Compt. rend. Soc. Biol., 151, 387-

Bowman, F., Merrison, S.M. & Stanek, M. 1955. Botulism and home canning.
Colo. Agr. Coll. Ext. Bull., nr 394-A, 11 p.

Brasmer, T.H. & Shideler, R.K. 1956. "Pseudobotulism" in dogs.
North. Amer. Vet., 37, 669-670.

Breitzke, C. 1941. Klinische Beobachtung über eine schwere familiäre Botl.-Intoxikation.
Med. Klin., 20, 507-510.

Briegor & Kempner. 1897. Beitrag zur Lehre von der Fleischvergiftung.
Deut. Med. Woch., 23, 521-

Brini, A. & Quéré, M.A. 1955. À propos de quelques cas de botulisme.
Rev. d'oto-neuro, 27, 237-238.

Brit. Med. J. 1957, p. 1537. Botulism.

Brocklehurst, J.C. 1957. /Botulism in Labrador./ Brit. Med. J., 2, 924.

Bronfenbrenner, J., Schlesinger, M.J. & Orr, P.F. 1921. Concerning toxic by-products of Bac. botulinus.
Proc. Soc. Exper. Biol. & Med., 18, 181-182.

Bronfenbrenner, J., Schlesinger, M.J. & Orr, P.F. 1924. Cause of immediate death by large doses of bot. toxin.
J. Exper. Med., 40, 81-89.

Brooks, V.B. 1953. Motor nerve filament block produced by bot. toxin.
Science, 117, 334-335.

Brooks, V.B. 1954. Action of bot. toxin on motor-nerve filaments.
J. Physiol., 123, 501-515.

Bruyère, A. & David, A. 1951. Paralysie labio-glosso-pharyngée et botulisme, chez le cheval.
Rev. Méd. Vét., 102. 155-160.

Brygoo, E.R. 1953. Action des agents épurateurs sur les toxines botuliniques en dilution dans l'eau.
Ann. Inst. Pastour., 84, 1039-1041.

Buehler, H.J., Schantz, E.J. & Lamanna, C. 1947. The elemental and amino acid composition of crystalline Cl.bot. type A toxin.
J. Biol. Chem., 169, 295-302.

Burgen, A.S.V., Dickens, F. & Zatman, L.J. 1949. The action of bot. toxin on the neuromuscular junction.
J. Physiol., 109, 10-24.

Bürger, L. 1953. Die Kornersche Krankheit, eine zunehmende Gefahr für die Volksgesundheit. (Botulism.)
München Med. Wschr., 95, 703-

Burkhart, R.L. 1957. Botulism in mink, a preventable disease.
Natl. Fur News, 29, no. 4, p. 11 & 30

Burrows, W., Gordon, F.B., Porter, R.J. & Moulder, J.W. 1949. Textbook of bacteriology.
pp. 591-596, Saunders, Philadelphia.

Buttiaux, R. 1955. Le difficile problème bactériologique des semiconserves de viandes en boîtes.
Antonie van Leeuwenhoek, 3, 267-276.

Byrne, K.V. 1952. Botulism in sheep./and cattle/.
Inst. Insp. Stock N.S. Wales. Ybk., 49, 51-52.

Cameron, E.J. & Bohrer, C.W. 1951. Food preservation with antibiotics: The problem of proof.
Food Techn., 5, 340-342.

Cammack, K.A. 1957. The significance of a second macromolecular component observed in preparations of Cl.bot. type A toxin.
Biochem. J., 67, 30-31.

Campbell, Jr., L.L. & Winiarski, W. 1959. Isolation and properties of a subtilin resistant strain of Cl.bot.
Appl. Microbiol., 7, 285-288.

Caparrini, W. 1959. Food poisoning from Cl.bot.
Zooprofilassi, 14, 407-414.

Caprio, F.S. 1936. An outbreak of botulism in New Jersey.
J.A.M.A., 106, 387-389.

Carda Aparici, P. 1955-56. Nuevas aportaciones al estudio del botulismo equino en España.
Madrid. Inst. Invest. Vet. An., 7, 173-191.

Cardella, M.A., Duff, J.T., Gottfried, C., & Bogel, J.S. 1956. Preparation of Cl. bot. type C toxoid from purified toxin.
Bacteriol. Proc., 90-

Cardella, M.A., Duff, J.T., Wingfield, B.H. & Gottfried, C. 1957. Production and purification of Cl.bot. type D toxin for conversion to toxoid.
Bacteriol. Proc., 93-

Cardella, M.A., Duff, J.T., Gottfried, C. & Bogel, J.S. 1958. Studies on immunity to toxins of Cl.bot. IV. Production and purification of type C toxin for conversion to toxoid.
J. Bacteriol., 75, 360-365.

Carlander, O. 1955. Fall av botulism. (Outbreaks of Botulism)
Sv. Läkartidningen, 52, 2918-2919.

Cartwright, T.E. & Lauffer, M.A. 1952. Assay of bot. A toxin with goldfish.
Proc. Soc. Exper. Biol. & Med., 81, 508-511.

Cartwright, T.E. & Lauffer, M.A. 1958. Temperature effect on botulinus A toxin.
Proc. Soc. Exper. Biol. & Med., 98, 327-330.

Castell, C.H. 1947. Growth of Cl. in seaweeds and marine fish.
J. Fisheries Res. Bd Can., 7, 62-69.

Castell, C.H. & Anderson, G.W. 1947. Effect of salting and smoking on survival and growth of Cl. in fish.
J. Fisheries Res. Bd Can., 7, 70-73.

Cheatum, E.L., Reilly, J.R. & Fordham, S.C. 1957. Botulism in game farm pheasants.
North Amer. Wildlife Conf. Trans., 22, 169-179.

Claronburg, A. & Fijen, J.N. 1937. Food poisoning by Bac. bot. type B.
Ned. T. Geneesk., 81, 4806-4813.

Claronburg, A. 1950. Bacteriecl Bodorf on Bosmetting van Voedingsmiddelen in Verband met de Volksgezondheid.
Voeding, 11, 329-340.

Clifton, C.E. 1939. Utilisation of amino acids by Cl.bot.
Proc. Soc. Exper. Biol. & Med., 40, 338-340.

Coleman, G.E. & Meyer, K.F. 1922. Some observations on the pathogenicity of Bac. bot. J. Infect. Dis., 31, 622-649.

Coleman, I.W. 1954. Studies on the oral toxicity of Cl.bot. toxin type A.
Can. J. Biochem. & Phys., 32, 27-34.

Colson, H. 1953. Recherches sur le botulisme d'origine pisciaire.
Thèse de Médecine, Paris.

Cook, E.W. 1945. The occurance of Cl.bot. and Cl. tetani in certain plots of soil.
Ohio State Univ. Abstracts.
Doctoral Diss.,47, 9-14.

Croisé. 1934. Le botulisme chez l'homme et les animaux. Diss. Paris.

Csiky, P. 1958. Botulismusfälle.
Arch. Tox., 17, 172-178.

Dack, G.M. 1926. The influence of some anaerobic species on the toxin of Cl.bot. with special reference to Cl. sporogenes
J. Inf. Dis., 38, 165-172.

Dack, G.M. & Gibbard, J. 1926. Studies on bot. toxin in the aliment. tract of hogs rabbits, guinea-pigs and mice.
J. Infect. Dis., 39, 173-180.

Dack, G.M. 1926. The lab. diagnosis of bot.
Am. J. Pub. Health, 16, 744-745.

Dack, G.M. & Wood, W.L. 1928. Serum therapy of botulism in monkeys.
J. Infect. Dis., 42, 209-212.

Dack, G.M. & Wood, W.L. 1928. The mechanism of toxin production by Cl.bot.
J. Infect. Dis., 42, 213-217.

Dack, G.M. & Williston, E.H. 1929. Effect of oxygen on the viability of young cultures of Cl.bot.
J. Infect. Dis., 44, 27-32.

Dack, G.M. & Hoskins, D. 1942. Absorption of bot. toxin from the colon of Macaca mulatta.
J. Infect. Dis., 71, 260-263.

Dack, G.M. & Wagennar, R.O. 1955. Effect of irradiation on the spores and toxin of Cl. bot.
Food Techn., 9, No. 5, sup., p. 28, ref. no. 102.

Dack, G.M. 1956. Food Poisoning.
Univ. Chicago Press, Chicago, 3rd edn. pp. 59-108.

Dahr, P. 1937. Bot. nach Konservengenuss.
Med. Welt, 11, 1331-

Dahr, P. 1941. Zur Frage der Hitzebeständigkeit des Botulismustoxins.
Med. Klin., 37, 1107-1108.

Dann. 1828. De veneni botulini viribus et natura.
Berolini, Inaugural Dissertation.

Dauer, C.C. 1952. Food and water borne disease outbreaks.
Publ. Health Rep., 67, 1089-1095.

Dauer, C.C. 1953. 1952 summary of food-borne, waterborne and other disease outbreaks. (Inkl. 2 små utbrott av botulism), Toxin typ A. (Included 2 Small Outbreaks of Botulism) Hemkonserverad svamp gav 3 sjuka och 2 döda. Hemkonserverade "beets" gav 2 sjuka som dog. (Home-canned mushrooms caused 3 ill and 2 dead. Home-canned "beets" caused 2 deaths.
Publ. Health Rep., 68, 696-702.

Dauer, C.C. & Sylvester, G. 1954. 1953 summary of disease outbreaks. (10 fall med 4 döda i bot. Orsak hemkonserver; grönsaker och hummerstjärt) Typ A och B. [(10 cases with 4 deaths due to botulism) Reason: home-canned preserves; vegetables, and lobster tail.] Publ. Health Rep., 69, 538-546.

Davies, J.R., Morgan, R.S., Wright, E.A. & Wright, G.P. 1953. Results of direct injections of bot. tox. into central nervous system of rabbits.
J. Physiol., 120, 618-623.

Davis, J.B., Mattman, L.H. & Wiley, M. 1951. Cl.bot. in fatal wound infection.
J.A.M.A., 146, 646-648.

Dodić, K. 1954. Botulismus beim Ners.
Mon. f. Vetmed., 9, 486-487.

Delaitre, Herman & Varlet. 1955. Syndrome de Guillain-Barré simulant un botulisme chez un enfant de 11 ans.
Rev. d'oto-neuro, 28, 189-192.

Demarchi, J., Mourgues, C., Orio, J. & Prévot, A.R. 1958. Existence du botulisme humain de type D.
Bull. Acad. Natl. Méd., 142, 580-582.

Denny, C.B., Bohrer, C.W., Perkins, W.E., Townsend, C.T., Pratt, G.B. & Wheaton, E. 1958. Destruction of Cl.bot. by ionising radiation I-II. (Abs.)
I. In neutral phosphate at room and freezing temperatures; II. Peas, chicken soup and pork in the frozen state.
Food Techn., 12, nr 4, sup. p. 39, ref. 90.

Denny, C.B. et al. 1959. Destruction of Cl.bot. by ionising radiation.
Food Res., 24, 44-50.

Derkach, V.N. 1956. The antitoxic properties of antibiotics. I. The detoxification of bot. toxin by antibiotics.
Trudy Khar'kov Nauch-Issledovatel Inst. Vaktsin i Syvorotok, 21, 25-33.

Derkach, V.N. 1956. The antitoxic properties of antibiotics. II. Experimental toxicosis therapy with combined antibiotics - and antibotulinus immune serum. Trudy Khar'kov Nauch.-Issledovatel Inst. Vaktsin i Syvorotok, 23, 35-42.

Dewberry, E.B. 1959. Food poisoning. p. 294-344. L. Hill Ltd. London.

Dickson, E.C., Burke, G.S., Beck, D., Johnson, J. & King, H. 1922. Studies on the thermal death time of spores of Cl.bot. Preliminar report. J.A.M.A., 79, 1239-1240.

Dickson, E.C. & Shovky, R. 1923. Botulism. Studies on the manner in which the toxin of Cl.bot. acts upon the body. I. The effect upon the autonomic nervous system. J. Exper. Med., 37, 711-731.

Dickson, E.C. & Shovky, R. 1923. Studies on the manner in which the toxin of Cl. bot. acts upon the body. II. The effect upon the voluntary nervous system. J. Exper. Med., 38, 327-346.

Dickson, E.C. 1928. Dormency and delayed germination of spores of Cl. bot. subject to heat. Proc. Soc. Exper. Biol. & Med., 25, 426-427.

Dimock, W.W. & Tyant, Z.N. 1949. Botulism in domestic animals. Cause-nature-diagnosis-and recommendations on prevention Kentucky Agr. Exp. Stat. Circ. No. 62.

Dinter, Z. & Kull, K.-E. 1950. Nord. Vet. Med., 2, 286-

Dinter, Z. & Kull, K.-E. 1951. Ergänsende Untersuchungen über den Botulismus beim Nerz. Nord. Vet. Med., 3, 297-311.

Dinter, Z. & Kull, K.-E. 1954. Über einen Ausbruch des Botulismus bei Fasanenküken. Nord. Vet. Med., 6, 866-872.

Dinter, Z. & Kull, K.-E. 1955. Einiges über den Botulismus beim Nerz. Deut. Tierärztl. Wchnschr., 62, 53-55.

Dinter, Z. & Kull, K.-E. 1955. Über die Empfänglichkeit des Nerzes für die Botulinus-Toxine A und C. Nord. Vet. Med., 7, 549-563.

Dinter, Z. 1955. Zur aktiven Immunität gegen das Bot. tox. C. Nord. Vet. Med., 7, 1056-1062.

Dische, F.E. & Elek, S.D. 1957. Experimental food-poisoning by Cl. welchii. Lancet, 2, 71-74.

Dobberstein, J. & Piening, C. 1933. Berl. Münch. tierärztl. Wschr., 49, 549-

Dolman, C.E. & Kerr, D.E. 1947. Botulism in Canada, with report of a type E outbreak at Nanaimo, B.C. Canad. J. Publ. Health, 38, 48-57.

Dolman, C.E. & Kerr, D.E. 1948. Canad. Med. Ass. J., 59, 412-

Dolman, C.E., Chang, H., Kerr, D.E. & Shearer, A.R. 1950. Fish-borne and type E botulism: Two cases due to home-pickled herring. Can. J. Publ. Health, 41, 215-229.

Dolman, C.E. & Chang, H. 1952. Can. J. Publ. Health, 43, 38-

Dolman, C.E. & Chang, H. 1953. The epidemiology and pathogenesis of type E and fish-borne botulism. Can. J. Publ. Health, 44, 231-244.

Dolman, C.E. 1953. Human botulism in Canada. Canad. Med. Ass. J., 68, 538-543.

Dolman, C.E. 1954. Additional botulism episodes in Canada. Canad. Med. Ass. J., 71, 245-249.

Dolman, C.E. 1954. Human botulism. Can. J. Publ. Health, 45, 403-406.

Dolman, C.E., Darby, G.E. & Lane, R.F. 1955. Type E botulism due to salmon eggs. Can. J. Publ. Health, 46, 135-141.

Dolman, C.E. 1957. Recent observations on type E botulism. Can: J. Publ. Health, 187-198.

Dolman, C.E. 1957. Human botulism and staphylococcal food poisoning: a review. Jap. J. M. Sc. Biol., 10, 373-381.

Dolman, C.E. 1957. Type E (fish-borne) botulism: a review. Jap. J. M. Sc. Biol., 10, 383-395.

Dolman, C.E. et al. 1960. Fish eggs as a cause of human bot. 2 outbreaks in B.C. due to types E and B bot. toxins. J. Infect. Dis., 106, 5-19.

Dozier, C.C., Wagner, E. & Meyer, K.F. 1924. Effect of glucose on biochemical activities, including growths and toxin production of B. botulinus. J. Infect. Dis., 14, 85-

Dozier, C.C. 1924. Optimum and limiting hydrogen-ion concentrations for B. botulinus and quantitative estimation of its growth. XVI.
J. Infect. Dis., 35, 105-133.

Dräger, K. et al. 1957. Immunisierungsversuche gegen Staupe und Botulismus bei Nerzen.
Tierärztl. Umschau, 12, 136.

Dubovsky, B J. & Meyer, K.F. 1922. An exp. study on the methods available for the enrichment, demonstration and isolation of B. bot. in specimens of soil and its products, in suspected food, in clinical and necropsy material
J. Infect. Dis., 31, 501-540.

Dubovsky, B.J. 1922. The distribution of the spores of B. bot. in the territory of Alaska and in the Dominion of Canada.
J. Infect. Dis., 31, 595-599.

Duff, J.T. et al. 1956. Activation of Cl. bot. type E toxin by trypsin.
J. Bact., 72, 455-460.

Duff, J.T., Wright, G.G., Klerer, J., Moore, D.E. & Bibler, R.H. 1957. Studies on immunity to toxins of Cl.bot. I. A simplified procedure for isolation of type A toxin.
J. Bact., 73, 42-47.

Duff, J.T., Klerer, J., Bibler, R.H., Moore, D.E., Gottfried, C. & Wright, G.G. 1957. Studies on immunity to toxins of Cl.bot. II. Production and purification of type B toxin for toxoid.
J. Bact., 73, 597-601.

Dukalov, I.A. 1958. Problem of intravital diagnosis of botulism.
Zh. Mikrob. (Moskva), 29, 43-46.

Dumoste, M.H. 1955. Botulisme equin.
Encyc. Vét. Période., 3, 55-58.

Edes, C.E. & Turner, A.W. 1952. Description of Cl.bot. type D recovered from soil in South Australia.
Australian J. Exptl. Biol. Med. Sci., 30, 295-300.

Edgar, G. 1950. Forage poisoning or botulism. N.S. Wales Dept. Agr. Div. Anim. Indus. Dis. Anim. L., no. 55, 3 p.

Edmondson, R.B., Giltner, L.T. & Thom, C. 1922. Bacillus botulinus: relation of toxin production to temperature.
Abstr. Bacteriol., 6, 23.

Edmondson, R.B., Thom, C. & Giltner, L.T. 1923. Experiments with B. bot. under household conditions.
Amer. Fd. J., 18, 143-

Edmunds, C.W. & Long, P.H. 1923. Contributions to the pathologic physiology of botulism.
J.A.M.A., 81, 542-547.

Ermengem, van. 1897. De l'etiologie du botulisme. Compte rend.
Soc. de biol., 10e ser., 4, 155.

Erzina, G.A., Mikhailov, V.V. 1956. The mechanism of the action of botulinus type A toxin on frog heart.
Bull. Exptl. Biol. Med., 41, 129-132.

Esselen, W.B. jr. 1945. Botulism and home canning, Amherst, Mass.
Mass. Agr. Exp. Stat. Bull. No. 426.

Esty, J.R. & Meyer, K.F. 1922. The heat resistance of spores of Bac. botulinus and allied anaerobes.
J. Infect. Dis., 31, 650-663.

Evans, F.R. & Curran, H.R. 1952. The preserving action of subtilin and mild heat in normal and concentrated milk.
J. Dairy Sci., 35, 1101-1106.

European Technical Conference on Food-Borne Infections and Intoxications.
World Health Organisation. Technical Report Series WO 184, 1959. Geneve.

Farleigh, E.A. 1949. Botulism in wild birds.
Yearbook of the Institute of Inspectors of Stock, New South Wales, p. 101.

Faust, M. 1952. Home canning, Berkeley, Calif. Agr. Ext. Serv. Coll. of Agr. Univ. of Calif.

Fleming, R.H. 1960. Report on 4 cases of botulism in Catocoma, S. Rhodesia.
Centr. Afr. J. Med., 6, 91-94.

Foster, J.W. & Wynne, E.S. 1948. Physiological studies on spore germination with special reference to Cl.bot. IV. Inhibition of germination by unsaturated C_{18} fatty acids. J. Bact., 55, 495-501.

Francillon, M. 1925. Einfluss der aeroben Mischinfektion auf Entwicklung und Toxinbildung des Bacillus botulinus. Arch. f. Hyg., 95, 121-

Fulthorpe, A.J. 1955. Cl. bot. types A and B toxin-antitoxin reactions. J. Hygiene, 53, 189-195.

Fåraeus, J. 1949. Botulinum bacilli and their occurence in Sweden. State Bact. Lab., Stockholm, 111 p.

Gabrys, K. & Szaflarski, J. 1959. Botulism in minks. (In Polish.) Med. Wet., 15, 40-43.

Gale, C. 1955. Comments on botulism, other mink diseases. U.S. Fur Rancher, 34, 4, 7, 15, 18.

Geiger, J.C., Dickson, E.C. & Meyer, K.F. 1922. The epidemology of botulism. Publ. Health Bull. no. 127.

Geiger, J.C. 1924. Amer. J. Publ. Health, 14, 309.

Geiger, J.C. 1941. An outbreak of botulism J. Amer. Med. Ass., 117, 22-

Generoso, J.D. & San Augustin, F. 1940. Philipp. J. Anim. Ind., 7, 419.

Gendon, Yu. Z. 1958. Studies on botulin antigens and antisera using methods of zone electrophoresis and agar diffusion precipitations. Zh. Mikrob. (Moskva), 29, no. 2, p. 95-100.

Gendon, Yu.Z. 1958. Study of proteins of type A botulinus toxin by paper electrophoresis. Voprosy Med. Khim., 4, 182-186.

Gendon, Yu.Z. 1958. Studies on antigenic composition of botulinus toxins and antitoxins A with the aid of diffusion precipitation reaction on agar. Zh. Mikrob. (Moskva), 29, no. 5, p. 33-37.

Gerbaut, P. et al. 1959. A propos of an epidemic of 6 cases of botulism. Rev. Med. Nancy, 84, 934-940.

Geurden, L.M.G. 1949. Botulism in man and animals. 14th. Int. Vet. Congr. Rpt.(publ. 1952), 1, 326-328.

Gibbs, B.M. & Hirsch, A. 1956. Spore formation by Cl. species in an artificial modium. J. Appl. Bact., 19, 129-141.

Giltner, L.T. & Couch, J.F. 1930. Western duck sickness and botulism. Science, 72, 660.

Gitter, M. 1959. Bot. in mink an outbreak caused by type C-toxin. Vet. Rec., 71, 868-871.

Glotowa, E. & Dankerowitz . 1936. Zur Standardisierung des Antibotulinus-Serums. J. f. Vet. Med., 58, 385.

Gold, E.R. & Ilian, I. 1957. Botulinus toxin A und Hämagglutininrezeptoren der roten Blutkörperchen. Zbl. Bakt. I. Orig., 167, 385.

Gonzales, C.D. & Martino, O.A. 1957. Botulismo. Dia méd. B. Aires, 29, 2221.

Gooding, D. 1959. The use of Botulinus toxin as a poison for kangaroo (marloo) control in W.A. Austral. Inst. Agr. Sci. J., 25, 141-143.

Gordon, M., Flock, M.A., Yarinsky, A. & Duff, J.T. 1957. Studies on immunity to toxins of Cl.bot. III. Preparation, purification and detoxification of type E toxin. J. Bacteriol., 74, 533-538.

Gorham, J.R. 1950. Reviewing botulism in mink. Fur J., 16, no. 6 p. 10.

Gorham, J.R. 1957. A case report of the botulism outbreak in the Northwest. Natl. Fur News, 29, no. 9, p. 12-13, 46-48.

Gorham, J. & White, P. More about botulism. Black Fox Mag. & Mod. Mink Breeder, 41, no. 7, p. 20, 37-38, 41.

Goosweiler, N. 1957. Protective action of dinitrile against poisoning with bot. toxin A. Arch. exptl. pathol. pharmakol., 231, 279-285.

Goosweiler, N. 1957. Effect of succinodinitril on the muscle poisoned with curare and botulinus toxin A. Arch. exptl. pathol. pharmakol., 231, 286-291.

Graham, R. & Schwarzo, H. 1921. Differentiation of type A and type B bot. toxins in food. A rapid and simple method.
J.A.M.A., 76, 1743-1744.

Gray, D.F. 1948. Med. J. Aust., 35, 37.

Grecz, N. Wagenaar, R.O. & Dack, G.M.1955. Effect of fatty acids in pasteurized Liederkranz cheese on the growth of Cl.bot. added to the product.
J. Dairy Sci., 38, 589.

Grecz, N., Wagenaar, R.O. & Dack, G.M. 1959. Inhibition of Cl.bot. and molds in aged, surface ripened cheese.
Appl. Microbiol., 7, 33-38.

Grecz, N., Wagenaar, R.C. & Dack, G.M. 1959. Relation of fatty acids to the inhibition of Cl.bot. in aged,surface ripened cheese.
Appl. Microbiol., 7, 228-234.

Greenberg, R.A. et al. 1958. Toxin production and organoleptic breakdown in vacuum packaged fresh meats inoculated with Cl.bot.
Food Res., 23, 656-661.

Greenberg, R.A. et al. 1959. The influence of sodium chloride on toxin production and organoleptic breakdown in perishable cured meat inoculated with Cl.bot.
Food Techn., 13, 509-511.

Greenleaf, C.A. 1955. Current status of cold sterilization.
N.C.A. Information Letter, no. 1538, p. 226-227.

Grieg, A. 1957. An outbreak of the disease in Rygge, Østfold county./Botulism./
Tidsskr. f. d. norske Lægef., 77, 738.

Gromakovskaia, M.M. 1957. Effects of tetanus and bot. toxins on chronaxy in immune and non immune rabbits.
Bull. eksp. biol. med., 43, 46-50.

Grotenhuis, G. 1950. A case of limbernock. /of poultry, caused by Cl.bot./ (In Dutch.)
Tijdschr. v. Diergeneesk, 81, 437-439.

Guenther, D. & Mather, A.M. 1952. Growth and toxin production by Cl.bot. in the presence of sulfhydryl compounds.
Proc. S. Dakota Acad. Sci., 31, 164.

Guillaumie, M. & Kreguer, A. 1951. Contribution á l'étude des hémolysine bacteriennes. Propriétés des toxines botuliniques des types C et D.
Compt. Rend. Soc. Biol., 145, 179-182.

Guillaumie, M. et al. 1952. Propriétés de diverse serums anti-gangréneux au cours de l'immunisation du cheval.
Ann.Inst. Past., 83, 360.

Guillaumie, M., Kreguer, A. & Geoffrey, M. 1955. Some properties of toxins produced by different types of Cl.bot.
Ann. Inst. Past., 88, 44-59.

Guillaumie, M., Kreguer, A. & Geoffrey, M. 1955. Action of different serums on bot. toxin.
Ann. Inst. Past., 88, 153-169.

Gunderson, M.F. 1935. Insect carriers of Cl.bot.
J. Bact., 30, 333.

Gunnison, J.B. & Meyer, K.F. 1928. Susceptibility of macacus rhesus monkey to bot. toxin type B, C and D.
Proc. Soc. Exper. Biol. & Med., 26, 89.

Gunnison, J.B. & Meyer, K.F. 1929. The occurrence of nontoxic strains of Cl. parabot. XXXIV.
J. Infect. Dis., 45, 79-85.

Gunnison, J.B. & Meyer, K.F. 1929. Cultural study of an international collection of Cl.bot. and parabot. XXXVIII.
J. Infect. Dis., 45, 119-134.

Gunnison, J.B. et al. 1932.
J. Infect. Dis., 51, 542.

Gunnison, J.B., Cummings, J.R. & Meyer, K.F. 1937. Cl.bot. type D.
Proc. Soc. Exper. Biol., N.Y., 35, 278-280.

Guyton, A.C. & MacDonald, H.A. 1947.
Arch. Neurol. & Psychiat., 57, 578-592.

Gültürk, S. 1954. Lamziekte caused by Cl. bot. type D in Turkish cattle. (In Turkish with German summary.)
Türk. Vet. Hekimleri Derneği, Dergisi, 24, 1612-1619.

Haerem, S., Dack, G.M. & Dragstedt, L.R. 1938. Acute intestinal obstruction. II. The permeability of obstructed bowel segments of dogs to Cl.bot. toxin.
Surgery, 3, 339-350.

Haines, R.B. 1942. The occurrence of toxigenic anaerobes esp. Cl.bot. in some English soils.
J. Hyg., 42, 324.

Hakioglu, F. 1957. Darmentzündungen beim Botulismus des Rindes.
Deut. Tierärztl. Wchnschr., 64, 421-423

Hakioglu, F. 1957. Erster Bericht über enzootisch verlaufenden Pferdebotulismus in der Türkei mit Scheinsymptomen einer Meningo-Encephalomyelitis.
Deut. Tierärztl. Wchnschr., 64, 79-81.

Hall, I.C. & Peterson, E.C. 1923. The effect of certain bacteria upon the toxin production of Bacillus bot. in vitro.
J. Bact., 8, 319-341.

Hall, I.C. 1933. Further outbreaks of bot. in the Rocky Mountain region.
Am. J. Hyg., 17, 235-251.

Hall, I.C. 1936. New outbreaks of bot. in West USA.
Food Res., 1, 171-198.

Hall, I.C. 1943. Danger of botulism.
Am. J. Publ. Health, 33, 818-820.

Hall, I.C. 1945. The occurrence of B. bot. types A and B in accidental wounds.
J. Bact., 50, 213-217.

Halliwell, G. 1954. Action of proteolytic enzymes on Cl. parabot. toxin.
Biochem. J., 58, 4-8.

Halvorsen, H.O. 1955. Factors determining survival and development of Cl.bot. in semipreserved meats.
Inst. Past. de Lille Ann., 7, 53-67.

Hampson, C.R. 1951. A case of probable botulism due to wound infection.
J. Bact., 61, 647-

Hansen, J.D. & Appleman, M.D. 1955. The effect of sorbic, propionic and caproic acids on the growth of certain Cl.
Food Res., 20, 92-95.

Harmsen, H. 1953. Botulismus durch Fischmarinaden.
Fischw.- u. Feinkostind., 25, 165-166.

Harmsen, H. 1954. Botulismus-Fischvergiftungen.
Dtsch. Lebensmitt. Rdsch., 50, 52-54 and 97-100.

Harmsen, H. 1955. Noch einmal: Botulismus-Fischvergiftungen.
Dtsch. Lebensmitt. Rdsch., 51, 196-197.

Hartwich, A. 1953. Lebensmitteltechnik besiegt eine Krankheit: die Gefahr des Botulismus ausgeschaltet.
Lebensm. u. Ernähr., 6, no. 2, 9-10.

Haugo, S. & Skulberg, A. 1955. The problem of mink botulism caused by offals from slaughterhouses.
Int. Ass. Vet. Food Hyg. Proc., 1, 93-95.

Hazen, E.L. 1937. A strain of B. bot. not classified as type A, B or C.
J. Infect. Dis., 60, 260-264.

Hazen, E.L. 1938. Incitants of human bot.
Science, 87, 413-414.

Hazen, E.L. 1942. Differential characters of 2 strains of Cl. bot. Type B: Action of toxins in chickens.
Proc. Soc. Exper. Biol. & Med., 50, 112-114.

Henrich, H. 1953. Erfolge mit Vit. B Injektionen bei Botulismus.
Med. Klin., 48, 279-

Herbert, J. 1946. Le Botulisme chez les animaux. Diss. Paris.

Hettche, H.O. & Strassburger, H. 1939. Die Wirkung v. Eisen, Mangan und Kupfersalzen bei letaler Tet. u. Bot. Intox. d. Maus.
Ztschr. f. Immunitätsforschung, 97, 109-120.

Heyningen, W.E., van. 1955. Recent developments in the field of bacterial toxins.
Schweiz Ztg. allgem. Pathol. u. Bakteriologie, 18, 1018-1035.

Heyningen, W.E., van. 1955. The role of toxins in pathology. Mechanisms of microbial pathogenicity.
5th Symposium Soc. Gen. Microbiol., 15, 17-39.

Hottle, G.A. & Abrams, A. 1947. Detoxification of crystalline bot. type A toxin
J. Immunol., 55, 183-187.

Hottle, G.A., Nigg, C. & Lichty, J.A. 1947. Studies on bot. toxoid, types A and B. II. Methods for determining antigenicity in animals. J. Immunol., 55, 255-262.

Howie, J.W. 1958. The bacteriology of food poisoning. Food & Nutr., Notes and Reviews.

Hunter, C.A., Weiss, J.E. & Olson, C.L. 1940. Botulism in South Dakota. Journal-Lancet, 60, 67-69.

Hupka, E. 1953. Gefahren der als Botulismus bekannten Futtervergiftung für die Haustiere. Tierzüchter, 5, 186-187.

Hitten, H. 1950. Verlustreiche Pferdeerkrankungen nach Futter, das durch Tierkadaver vergiftet war. Hannoversche Land- und Forstwirtschaft Ztg., 103, 673.

Iida, H., Nahamura, Y. et al. 1958. Additional type E bot. outbreaks in Hokkaido, Japan. Jap. J. M. Sc. Biol., 11, 215-222.

Ingram, M. & Robinson, R.H.M. 1951. The growth of Cl.bot. in acid bread media. Proc. Soc. App. Bact., 14, 62-72.

Ingram, M. & Robinson, R.H.M. 1951. A discussion of the literature on botulism in relation to acid foods. Proc. Soc. App. Bact., 14, 73-84.

Ingram, M. 1951. The effect of cold on microorganisms in relation to food. Proc. Soc. App. Bact., 14, 243-260.

Ingram, M., Handford, P.M. 1957. The influence of moisture and temperature on the destruction of Cl.bot. in acid bread. J. Appl. Bact., 20, 442.

Itô, T. & Satô, Y. 1957. Production of high potent botulinal type E toxin from resting cells with enzymic digestion. Jap. J. Vet. Res., 5, 1-2.

Jackson, R.F. 1953. Simultaneous occurrence of botulism in dogs and chickens. Vet. Med., 48, 509-510.

Jacquot, J. & Habert, H. 1943. Etude d'une petite enzootie de méningoencéphalomyélite du cheval dans la meme exploitation. Cahier. Méd. Vét., 13, 33.

Jacquot, J. & Prévot, A.-R. 1951. Recherches sur le botulisme équin expérimental. Ann. Inst. Past., 81, 334.

Jacquot, J. & Prévot, A.-R. 1952. Le botulisme expérimental du cheval provoqué par la toxine D. Evolution et essai de sérothérapie. Bull. Acad. Vét., 25, 247-253.

Jacquot, J. & Prévot, A.-R. 1953. Le botulisme expérimental du cheval provoqué par la toxine D. Bull. Acad. Vét., 26, 135-142.

Jacquot, J. 1954. Sur le botulisme équin et notamment le botulisme expérimental provoqué á l'aide de la toxine. Off. Int. des Epiz. B., 42, 473-481.

Jagtiani, J.K. 1951. Curried vegetables and Cl.bot. Ind. Food Pack, 5, no. 12, 11-12.

Jensen, L.B. 1926. Some infl. of antitoxic & other serums on bot. intoxications. J. Infect. Dis., 39, 413-424.

Jensen, L.B. 1944. Microbiol. problems in the preservation of meats. Bact. Revs., 8, 161-188.

Jensen, L.B. 1954. Microbiology of meats. 3 ed. Champaign, Ill. Garrard Press, p. 309-317, 320-344 & 403-404.

Jensen, L.B. 1956. Bacterial food poisoning. Food Drug and Cosmetic Law J., 11, 631-641, 644.

Jones, M.T. & Tanner, F.W. 1945. Incidence and distribution of Cl.bot. in soils of Ill. Food Res., 10, 238-245.

Jordan, E.O. & Dack, G.M. 1924. The effect of Clostridium sporogenes on Cl.bot. J. Infect. Dis., 35, 575-580.

Judo, A., Girard, P. & Carrat, P. 1949. Destructive action in vitro of certain chemical agents on botulinus and tetanus toxins. Compt. Rend. Soc. Biol., 143, 318-319.

Kadavy, J.L. & Dack, G.M. 1951. The effect of experimentally inoculating canned bread with spores of Cl.bot. and Bacillus mesentericus.
Food Res., 16, 328-337.

Kallstenius, B. 1935. Fall av botulism. (Cases of Botulism). Sv. Läkar-tidn., 32, 609-613

Kalmbach, E.R. 1930. Western duck sickness produced experimentally.
Science, 72, 658-660.

Kalmbach, E.R. 1935. Wird Bot. eine weitverbreitete Gefahr f.d. Wildgeflügel?
J. Am. Vet. Ass., 87, 183.

Kalmbach, E.R. 1938. Botulism, a recurring hazard to waterfowl.
US Dep. Agr. Bur. Surv. Wildlife Research Leaflet BS-120.

Kalmbach, E.R. 1939. Empfindlichkeit d. türk. Geiers gegen Bot.-Toxin.
J. Am. Vet. Ass., 94, 137-141.

Kalmbach, E.R. 1939. American vultures and the toxine of Cl.bot.
J. Am. Vet. Ass., 94, 187-191.

Kaplan, A.M. et al. 1954. Significance of variations in observed slopes of thermal death time curves for putrefactive anaerobes.
Food Res., 19, 173-181.

Karnitskaya, N.V. et al. 1956. Effect of carbon dioxide on botulin-producing bacteria in heat-smoked fish.
Voprosy Pitaniya, 15, no. 2, 49-50.

Katić, R.V. 1952. Sur une technique d'extraction de la toxine Cl.bot. (C) a partir des corps microbiens.
Rev. immunol., 16, 384-389.

Katić, R.V. 1952. Possibility of peroral active immunization of exp. animals with toxin of Cl.bot.
Rev. immunol., 16, 309-

Katić, R.V. 1953. Les possibilités d'apparition des infections botuliniques bytypes.
Cong. Internaz. di Microbiol. Riassunti delle Comun., 6, no. 3, 93-

Katić, R.V. 1954. Examen de la possibilité d'appliquer la méthode de l'hémagglutination pour déterminer Cl.bot. des types C et D.
Off. Internatl. des Epiz. B., 42, 496-506.

Katić, R.V. 1956. Lab.untersuchungen über die Wirkung des D Tubocurarine chlorides (1 %) auf intoxikation mit Cl.bot. Cβ toxine.
Schweiz Archiv. f. Tierheilk., 98,244-

Katić, R.V. & Dsinleski, G.B. 1956. Active substances of B. subtilis (RR) used in the treatment of experimental botulism caused by botulinum C β. Results of laboratory tests. (In Serbo-Croatian)
Veterinaria (Sarajevo), 5, 656-659.

Katić, R.V. 1956. Ergebnisse vergleichender experimenteller Untersuchungen über die therapeutische Wirkung von Antitoxin, Formoltoxoid und die Kombination beider auf die durch Cl.bot. D-Toxin hervorgerufene Intoxikation.
Zentralbl. Bakt. Abt., 1, Org., 166, 510-516.

Katić, R.V. 1956. Determination of stability of Cβ toxin of Cl.bot. (In Serb.)
Higijena (Beograd), 8, 273-276.

Katić, R.V. 1957. Laboratory studies on pathogenesis of botulism produced by Cl.bot.
Wien Klin. Wschr., 69, 211-213.

Katić, R.V., Puhac, I. & Mrgovic, N. 1957. Testing of the toxin of Cl.bot. C β on its stability in silage-infusions. (In Sorbo-Croatian with Engl. summary.)
Vet. Arh., 27, no. 5/6, 121-128.

Katić, R.V. 1957. Bacteriological diagnostics of botulism. (In Serbo-Croatian with Engl. summary.)
Veterinaria (Sarajevo), 6, 150-154.

Katić, R.V. et al. 1958. Possibility of using a bivalente vaccine against botulism and tetanus.
Rev.d'Immunol., 22, 51-

Kaufmann, O.W., Ordal, Z.J. & El-Bisi, H.M. 1954. Effect of several antibiotics on certain sporeforming organisms involved in food spoilage.
Food Res., 19, 483-487.

Kayukova, N.I. & Kremer, T.A. 1940. Development and toxin formation of Bacillus bot. in mixed culture.
Mikrobiologiya, 9, 585-593.

Kegeles, G. 1946. The molecular size and shape of bot. toxin.
J. Am. Chem. Soc., 68, 1670.

Kempe, L.L., Craikoski, J.T. & Gillies, R.A. 1954. Gamma ray sterilization of canned meat previously inoculated with anaerobic bacterial spores. Appl. Microbiol., 2, 330-332.

Kempe, L.L. et al. 1955. Combined effect of heat and radiation in food sterilization. Appl. Microbiol., 3, 346.

Kempe, L.L., Craikoski, J.T. & Bonventre, F.F. 1957. Combined irradiation-heat processing of canned foods. I. Cooked ground beef inoculated with Cl. bot. spores. Appl. Microbiol., 5, 292-295.

Kempe, L.L. et al. 1958. Combined irradiation-heat processing of canned foods. II. Raw ground beef inoculated with spores of Cl.bot. Appl. Microbiol., 6, 261-263.

Kempe, L.L. et al. 1959. Combined irradiation-heat processing of canned foods. III. Cooked ground beef inoculated with spores of a putrefactive anaerobe. Appl. Microbiol., 7, 131-134.

Keppie, J. 1951. The pathogenicity of the spores of Cl.bot. J. Hyg., London, 49, 36-45.

Korn, P. 1938. 2 Fälle einer schweren Bot.-Inf. Z. f. Fl. u. Milchhyg., 48, 180.

Korner. 1755, 1789, 1820. Neue Beobachtungen über die in Würtemberg so haufig vorfallenden todtlichen Vergiftungen durch den Genuss geraucherter Wurste. Tubingen, 1820.

Kerner. 1822, 1824. Das Fettgift, oder die Fettsaure, und ihre Wirkungen auf den thierischen Organismus. Ein Beytrag zur Untersuchung des in verdorbenen Wursten giftig wirkenden Stoffes. Stuttgart & Tubingen, 1822.

Kietzmann, M. 1957. Beobachtungen bei Tierversuchen zum Nachweis von Bot. Toxin. Berl. u. Münch. Tierärztl. Wschr., 70, 366-368.

Kindler, S.H. et al. 1955. Toxin production of resting cells of Cl. parabot. type A. Science, 122, 926-927.

Kindler, S.H. et al. 1956. Toxin production by Cl. parabot. type A. J. Gen. Microbiol., 15, 394-403.

Kindler, S.H. et al. 1956. Nutr. studies with the Cl.bot. group. J. Gen. Microbiol., 15, 386-393.

Knock, G.G. 1952. Survey of soils for spores of Cl.bot. (Union of S. Africa and SW. Africa) J. Sci. Food & Agr., 3, 86-91.

Knock, G.G. & Lambrechts, M.S.J. 1956. A note on the heat resistance of a S. African strain of Cl.bot. type E. J. Sci. Food & Agr., 7, 244-248.

Knothe, H. 1948. Über Botulismus. Aerztl. Wochenschr., 3, 534-535.

Korolova, G.A. et al. 1958. Prod. in horses of bivalente and polyvalente antibotulin sera A, B, C and E. Zh. Mikrob. (Moskva), 29, no. 5, 83-87

Koroleva, G.A. 1958. Prod. on horses of therapeutic antibotulic sera C and E. Zh. Mikrob. (Moskva), 29, no. 3, 102-106.

Krasnov, I. Jann, G.J. & Sallo, A.J. 1953. The effect of pH and heat on the activity of subtilin against spores of Cl.bot. Bacteriological Proceed., 53, 28.

Kravchenko, A.T. et al. 1959. Experience with production of antibotulin serums in cattle. Zh. Mikrob. (Moskva), 30, no. 4, 79-87.

Kuhn. 1824. Versuche und Beobachtungen über die Kleesaure das Wurst und das Kasegift, Leipsic.

Kull, K.E. & Moberg, K. 1952. Die Häufigkeit des Botulismus bei Futtervergiftungen des Nerzes in Schwoden. English summary. Nord. Vet. Med., 4, 771-779.

Kull, K.E. 1952. On botulism in mink. (In Swedish) Våra Pälsdjur, 23, 172-175.

Kull, K.E. 1953. Botulism of mink. Våra Pälsdjur, 24, 130-133.

Kull, K.E. 1956. Feed poisoning and vaccination /of minks against botulism./ Våra Pälsdjur, 27, 113-115.

Lamanna, C., MacElroy, O.E. & Eklund, H.W. 1946. The purification and crystallization of Cl.bot. type A toxin.
Science, 103, 613-614.

Lamanna, C. & Lewis, C. 1946. An observation of apparent substitution of pantothenate by thiamine and choline. (Cl. bot.)
J. Bact., 51, 390-399.

Lamanna, C. et al. 1946. Botulinum toxin (type A) including a study of shaking with chloroform as a step in the isolation procedure.
J. Bact., 52, 1-13.

Lamanna, C. & Glassman, H.N. 1947. The isolation of type B bot. toxin.
J. Bact., 54, 575-584.

Lamanna, C. & Dock, B.W. 1948. The antigen-antibody reaction with type A bot. toxin.
J. Immunol., 59, 231-247.

Lamanna, C. 1948. Haemagglutination by bot. toxin.
Proc. Soc. Exp. Biol. Met., 69, 332-336.

Lamanna, C. & Lowenthal, J.P. 1951. The lack of identity between Hemagglutinin and the toxin of type A bot. organism.
J. Bact., 61, 751-752.

Lamanna, C. & Jenson, W.I. 1952. Hemagglutination by Cl.bot. type C.
Bacteriological Proceedings, 52, 106.

Lamanna, C. et al. 1955. Body weights as factors in response of mice to botulinal toxins.
Am. J. Hyg., 62, 21-28.

Lamanna, C. 1959. The most poisonous poison.
Science, 130, 763-772.

Lehman, A.J. 1960. Quarterly report to the editor on topics of current interest. Botulism.
Ass. Food & Drug Off., 24, 147-149.

Lamanna, C. et al. 1960. Influence of ingested foods on the oral toxicity in mice of crystalline botulinal type A toxin.
J. Bact., 79, 406-410.

Lamarre, L. & Lamarre, H. 1944. Contribution à l'étude clinique du botulisme du cheval.
Bull. Acad. Vét., 17, 117-126.

Landmann, G. 1904. Über die Ursache der Darmstädter Bohnenvergiftung.
Hyg. Rundschau, 14, 449.

Lapčević, E., Konić, Lj., Pribičević, S. & Katić, R.V. 1954. Botulin intoxication in horses. (In Serbo-Croatian with Engl. summary.)
Veterinaria (Sarajevo), 3, 69-80.

Lapčević, E. 1954. L'intoxication des chevaux par le botulisme.
Off. Internatl. des Epiz. B., 42, 507-513.

Larson, A.E., Nicholes, P.S. & Gebhardt, L.P. 1955. Successfull immunization of mink with a toxoid against Cl.bot. type C.
Am. J. Vet. Res., 16, 573-575.

Larsen, A.E. 1956. Found: a prevention against botulism.
Am. Fur Breeder, 29, no. 2, p. 12-13, 54, 56.

Larsen, A.E. 1956. The second year of vaccination against botulism./Minks./
Am. Fur Breeder, 29, no. 4, p. 11-

Larsen, A.E. 1957. Control of botulism in mink.
Natl. Fur News, 29, no. 7, p. 18-

Larsen, A.E. 1958. Botulism, its relationship to mink nutrition.
Natl. Fur News, 30, no. 4, p. 40.

Lauffer, M.A. & Taylor, N.W. 1953. The influence of composition of the medium upon the sedimentation rate of proteins III. Crystalline botulinum A toxin protein.
Arch. Biochem., 43, 374-377.

LeBlanc, F.R., Devlin, K.A. & Stumbo, C.R. 1953. Antibiotics in food preservation. I. The influence of subtilin on the thermal resistance of spores of Cl.bot. and the putrefactive anaerobe 3679.
Food Techn., 7, 181-184.

Lecht, M. 1956. Botulismus beim Nerz.
Arch. Geflügelzucht u. Kleintierk., 5, 204-208.

Legroux, R. & Jeramec, C. 1935. Etudes sur la toxine et l'antitoxine botuliques.
C. r. Soc. Biol., 120, 641-643.

Legroux, R. Jeramec, C. & Segond, L. 1947. Destruction of bacterial toxins by proteases produced by microorganisms.
Ann. Inst. Past., 73, 828-830.

Leifson, E. 1931. Bacterial spores.
J. Bact., 21, 331-356.

Leighton, G. 1923. Botulism and food preservation. London: W. Collins.

Lemetayer, E., Nicol, L., Girard, O., Corvazier, R. & Choyroux, M. 1953. Recherches sur le botulisme expérimental chez le cheval.
Acad. Vét. de France. B., 26, 391-399.

Lewis, K.H. & Hill, E.V. 1947. Practical media and control measures for producing highly toxic cultures of Cl.bot. type A.
J. Bact., 53, 213-230.

Lewis, J.C., Michener, H.D., Stumbo, C.R. & Titus, D.S. 1954. Antibiotics in food processing. Additives accelerating death of spores by moist heat.
J. Agr. & Food Chem., 2, 298-302.

Lind, C.S. 1950. Studies on the growth of Clostridia.
14. Int. Dairy Congr. Proceedings.
Vol. 3, part 2, p. 276.

Littauer, U. 1951. Type A toxin of Cl.bot.
Nature, 167, 994-995.

Longo, D. 1936. Sulla specificità della prova biologica col siero di sangue per la diagnosi di botulismo.
Policlinico (soz. med.), 43, 477-483.

Lopez Garcia, E. et al. 1956. Botulism case with recovery.
Rev. Clin. Espan., 60, 393-

Lowenthal, J.P. & Lamanna, C. 1951. Factors affecting the botulinal hemagglutination reaction, and the relationship between hemagglutinating activity and toxicity of toxin preparations.
Am. J. Hyg., 54, 342-353.

Lubertino, J. 1957. Cl.bot. type A found in food preserved in curing brine.
Internatl. Symp. Food Microbiol. Proc., 2, 283-288.

Lund, A.J., Jansson, P.W. & Anderson, L.E. 1957. Effect of culture filtrates on sporogenesis in a species of Clostridium
J. Bact., 74, 577-583.

Lunde, S. 1948. Botulisme.
Ti. norske Laegeforening, 68, 133-136.

Lunde, S. 1955. Botulisme.
Ti. norske Laegeforening, 75, 522-526.

Lunde, S. 1959. Botulismo.
Liv og Helse, 26, 175-178.

MacKay-Scollay, E.M. 2 cases of botulism.
J. Path. Bact., London, 75, 482-485.

MacKee, M.T. 1955. Bacterial culture with controlled pH.
Appl. Microbiol., 3, 355-360.

MacKee, M.T., Bell, J.F.& Meyer, B.H. 1958. Culture of Cl.bot. type C with controlled pH.
J. Bact., 75, 135-142.

MacLachlan, J.J. & Newton, L.G. 1952. Botulism in poultry.
Queensland Agr. J., 74, 87-92.

Magee, H.E. 1953. Notes on the nutritional and therapeutic values of vegetable juices.
Mon. Bull. Minist. Health Lab. Serv., 12, 78-84.

Mager, J., Kindler, S.H. & Grossowicz, N. 1954. Nutritional studies with Cl. parabot. type A.
J. Gen. Microbiol., 10, 130-141.

Magoon, C.A. 1932. Microorganisms as affecting frozen foods.
Ind. Eng. Chem., 24, 669-

Mandia, J.W. 1951. The serological group II of the proteolytic clostridia.
J. Immunol., 67, 49-55.

Mandia, J.W. 1952. The role of heat-stable antigen in serological identification of prot. clostridia.
J. Immunol., 69, 451-455.

Maschor, W. & Gabinus, O. 1933. Om botulismus och ichthyismus neuroparalyticus.
Hygiea, 95, 1-10.

Matheson, B.H. et al. Diagnosis of botulism and methyl alcohol poisoning.
Canad. M.A.J., 72, 139.

Matrozova, R.G. 1956. Effect of tomatoes and tomato products on the spore development and toxin formation in Bac.bot. (In Russian.)
Krasnodar. Vsesojuz. Nauch. Issled. Inst. Konserv. i Ovoschchehessuschiljnoi Promyshl. Trudy., 6, 111-123.

Matsuoka, T. 1959. Equine botulism.
Nest. Vet., 6, 24.

Matveev, K.I., Bulatova, T.I. & Sergeeva, T.I. 1958. Immunization of minks against botulism. (In Russian with Engl. summary.)
Veterinaria, 35, no. 8, p. 42-45.

May, A.J. & Whaler, B.C. 1958. The absorption of Cl.bot. type A toxin from the alimentary canal.
Brit. J. Exptl. Pathol., 39, 307-316.

Meisel, H., Rybicka, I. & Zaleska, H. 1952. Botulinus type A toxin I.
Med. Doświadczalna i Mikrobiol., 4, 165-176.

Meisel, H., Rybicka, I. & Zaleska, H. 1952. Botulinus type A toxin II. Detoxification of the antigenicity.
Med. Doświadczalna i Mikrobiol., 4, 519-529.

Meyer, E.A. & Lamanna, C. 1959. Activity of type A botulinal toxin and hemagglutinin exposed to proteolytic enzyme.
J. Bacteriol., 78, 175-180.

Meyer, K.F. & Dubovsky, B.J. 1922 a. The distribution of spores of Bac.bot. in California.
J. Infect. Dis., 31, 541-555.

Meyer, K.F. & Dubovsky, B.J. 1922 b. The distr. of spores of Bac. bot. in the USA.
J. Infect. Dis., 31, 559-594.

Meyer, K.F. & Dubovsky, B.J. 1922 c. The occurrence of spores of Bac.bot. in Belgium, Denmark, England, the Netherlands and Switzerland.
J. Infect. Dis., 31, 600-609.

Meyer, K.F. & Gunnison, J.B. 1928. Cl.bot. type Sp. v.
Proc. Soc. Exp. Biol. & Med., 26, 88-89.

Meyer, K.F. 1929. Maximum oxygen tolerance of Cl.bot. types A, B & C of Cl. sporogenes and Cl. welchii.
J. Infect. Dis., 44, 408-411.

Meyer, K.F. & Gunnison, J.B. 1929 a. European strains of Cl.bot. XXXVI.
J. Infect. Dis., 45, 96-105.

Meyer, K.F. & Gunnison, J.B. 1929 b. South African cultures of Cl.bot. and parabot. XXXVII.
J. Infect. Dis., 45, 106-118.

Meyer, K.F. & Gunnison, J.B. 1929 c. Botulism due to home canned Bartlett pears. XXXIX.
J. Infect. Dis., 45, 135-147.

Meyer, K.F. 1931 a. Protective measures of State of California against botul.
J. Prevent. Med., 5, 261-293.

Meyer, K.F. 1931 b. Newer knowledge on botulism and mussel poisoning.
Am. J. Publ. Health, 21, 762-770.

Meyer, K.F. & Eddie, B. 1950. Fifty years of botulism in U.S. and Canada.
Mimeogr. rep. from C.W. Hooper Found. Univ. of Calif.

Meyer, K.F. & Eddie, B. 1951. Perspectives concerning botulism.
Zeitschr. f. Hygiene, 133, 255-263.

Meyer, K.F. 1953. Medical progress, food poisoning.
New Engl. J. Med., 249, 765-773, 804-812 & 843-852.

Meyer, K.F. et al. 1953. Cl.bot. type C and human botulism.
Proc. VI. Int. Microb. Congr. Rom, 4, 123-124.

Meyer, K.F. 1956. The status of botulism as a world health problem.
Bull. World Health Organization, 15, 281-298.

Meyer, R. 1950.
Mh. Vet. Med., 5, 13-15.

Mikhaylov, V.V. 1956 a. Site of action of toxin on interneural connections of the spinalcord.
Arkh. Patol., 18, no. 4, p. 29-34.

Mikhaylov, V.V. 1956 b. Mechanism of impairment of parasympathetic innervation of heart during exp. intoxication with bot. type A toxin.
Bull. Exp. Biol. & Med., 41, 45-47.

Miklausic, B., Zaharija, J. & Audi, S. 1951. L'epizootie de la paralyse spinale et bulbaire (botulisme) des chevaux sur une ferme. (In Serbo-Croatian with French summary.)
Vet. Arh., 21, 554-571.

Miller, B.E. 1957. Botulism and gastrotomy in the canine.
Seoast. Vet., 9, no. 2, p. 42-43, 66.

Millonig, R.C. 1956. Isolation of an aminopeptidase from type B Cl.bot.
J. Bact., 72, 301-307.

Minervin, S.M. 1957. Res. of several years of investig. on botulism.
Zh. Mikrob. (Moskva), 28, 30-35.

Moberg, K. 1953. Über die Festellung und Bekämpfung des Botulismus beim Nerz in Schweden.
Proc. 15. Intern. Vet. Congr. (Stockholm). Vol. 1, p. 62-64. Disc. in Vol. 2, p. 166-167.

Momberg-Jørgensen, H.C. 1952. Botulism of mink.
Dansk Pelsdyravl, 15, 352-353.

Momberg-Jørgensen, H.C. 1956. How to avoid botulism.
Dansk Pelsdyravl, 19, 231-234.

Moll, T. & Brandly, C.A. 1951. Botulism in the mouse, mink et ferret with special reference to suscoptibility and pathological alterations.
Am. J. Vet. Sci., 12, 355-363.

Morgan, B.H. & Reed, J.M. 1954. Resistance of bacterial spores to gamma radiation.
Food Res., 19, 357-366.

Morse, R.E., Powers, J.J. & Williams, C.J. 1950. Spores of Cl.bot. in Georgia soil.
Food Res., 15, 454-458.

Moses, H.E. 1952. Botulism in poultry.
Purdue Univ. Agr. Ext. L. no. 347, p. 4.

Mossel, D.A.A. et al. 1956. Enumeration of anaerobic bacteria, and of Clostridium species in particular, in foods.
J. Appl. Bact., 19, 142-154.

Mouren, P. et al. 1955. Fatal form of human botulism.
Bull. et mém. Soc. méd. hôp. Paris, 71, 723-726.

Mundt, W. 1956. Untersuchungen über Cl.bot.
Prakt. Tierarzt, 5, 125-126, 129.

Muromcew, S. 1938. Pferdebotulismus.
Sovjet. Vet., 15, no. 11, p. 23-25.

Murrell, W.G., Olsen, A.M. & Scott, W.J. 1950. The enumeration of heated bacterial spores. II. Experiments with bacillus species.
Austral. J. Sc. Res., Ser. B., 3, 234-244.

Müller. 1869. Das Durstgift.
Deutsch. Klin., 21, 321-
Ibid. 1870, 22, 27-

Müller, V. & Scheibel, I. 1960. Preliminary report on the isolation of an apparently new type of Cl.bot.
Acta Path. et Micr. Scand., 48, 80-

Nakamura, Y. et al. 1956. Type E botulism in Hokkaido, Japan.
Jap. J. Med. Sc. et Biol., 9, 45-58.

Natvig, H. 1955. Botulismo i Norge.
Ti. f. den norske laegeforening, 75, 538-559.

Nicholson, J.H. 1959. Botulism occurring as an isolated case.
North Carolina Med. J., 20, 146-149.

Nickerson, J.T.R., Proctor, B.E. & Goldblith, S.A. 1958. Freezing and irradiation.
Am. J. Publ. Health, 48, 1041-1048.

Nigg, C. et al. 1947. Studies on botulinum toxoid types A and B. I. Production of alun precipitated toxoids.
J. Immunol., 55, 245-254.

Novotna-Uzunova, M. 1956. 2 cases of botulism.
Suvrem. Med. Sofia, 7, no. 10, 128-130

Noyan, A. 1958. The value of urinanalysis in bovine botulism.
Am. J. Vet. Res., 19, 840-841.

Nunes Salvador, A.R. 1952. Botulism. (In man) (In Portuguese).
Vida Agr., 14, 2-3.

Næss, K. & Sögnen, E. 1958. Continued action of di-isopropylfluorophosphate and bot. toxin on the rat diafragm preparation.
Acta Pharmacol. Toxicol., 14, 333-340.

Oakley, C.L. 1956. Classification and biochemical activities of the genus Clostridium.
J. Appl. Bact., 19, 112-116.

Ohye, D.F. & Scott, W.J. 1953. The temperature relations of Cl.bot. types A and B.
Austr. J. Biol. Sci., 6, 170-189.

Ohye, D.F. & Scott, W.J. 1957. Studies in the physiology of Cl.bot. type E.
Austr. J. Biol. Sci., 10, 85-94.

Olsen, A.M. & Scott, W.J. 1950. The enumeration of heated bacterial spores. I. Experiments with Cl.bot. and other species of Cl.
Austr. J. Sci. Res. Ser. B, 3, 219-233

Onur, D.T. 1940. Observations on the antigenicity of alum-precipitated Cl.bot. type C toxoid.
Austr. Vet. J., 16, 4-13.

Pamukçu, A.M. 1954. Bovine botulism due to Cl.bot. type D in Turkey; toxin resulted in hemorrhagic encephalomyelitis (In Turkish with English summary.) Türk. Vet. Hekimleri Dernergi. Dergisi, 24, 1711-1755.

Pamukçu, A.M. 1954. Hemorrhagic encephalomyelitis due to Bot. in cattle in Turkey Zbl. Vet. Med., 1, 707-

Parry, E.W. 1946. Prevalence of Cl.bot. in the soils of central N.Y. state. Food Res., 11, 203-209.

Parsons, J.L. Botulism; Infrequent but lethal. N. Dak. Agr. Expt. Sta. Bimon. Bull., 20, no. 7, 13-15.

Pedersen, H.O. 1953. Botulism. Nord. Vetmed., 5, 709-730.

Pedersen, H.O. 1954. Cl.bot. type E. 7. Nord. Veterinaermøte. Saermøte H. Rapport 3, p. 472-489.

Pedersen, H.O. 1955. On type E botulism. J. Appl. Bact., 18, 619-629.

Pedersen, J. & Christensen, A. 1952. Shock therapy in botulism treated with dekstran and blood. Lancet, 2, 560-562.

Pedersen, H.O. 1957. The survival of Cl. bot. in curing brines. Proc. of the 2nd int. sympos. on food microbiology, Cambridge 1957, p. 289-293

Perry, H. Townsend, C.T., Andersson, A.A. & Berry, J.A. 1940. Studies on Cl.bot. in frozen pack vegetables. Food Techn., 2, 180-190.

Petersen, P.C. 1957. Botulismus i svinebesätning. (Botulism in hog litters) Medl.blad. dansko dyrlageforening, 40, no. 4, p. 69-72.

Pfenninger, W. 1924. Toxico-immunologic and serologic relationship of B. bot. type C, and B. parabot. "Soddon" XXII. J. Infect. Dis., 35, 347-352.

Phillips, E. & Reedy, L.M. 1953. Botulism in turkeys. South-West. Vet., 6, 272.

Pillai, C.P. & Kulasegaram, P. 1956. Suspected botulism in cattle in Ceylon. Ceylon Vet. J., 4, no. 3, p. 78-80.

Pitre, J. & Quittet, P.L. 1958. Étude pratique du pouvoir immunisant du vaccin antibotulinique "C" chez le vison. Acad. Vét. de France. B., 31, 379-382.

Pogue & Poole, . 1956. Pseudobotulism /case of a dog/. Auburn Vet., 13, 53, 58.

Polson, A. & Sterne, M. 1946. Production of potent botulinum toxins and formoltoxoids. Nature, 158, 238.

Pratt, C.B. et al. 1959. Destruction of Cl.bot. by ionizing radiation. II. Peas, chicken soup and pork in the frozen state. Food Res., 24, 51-56.

Prescott, S.C. & Geer, L.P. 1936. Food-poisoning organisms under refrigeration conditions. Refrigerating Eng., 32, 211-212.

Prévot, A.-R. 1938. C.R. Soc. Biol., 127, 685.

Prévot, A.-R. & Raynaud, M. 1944. C.R. Acad. Sc., 218, 126.

Prévot, A.-R. & Rossi, P. 1946. Bull. Acad. Vét., 19, 312.

Prévot, A.-R. 1947. Ann. Inst. Past., 73, 222.

Prévot, A.-R. et al. 1948. Bull. Acad. Vét., 21, 386-

Prévot, A.-R. & Brygoo, E.R. 1949. Étude de la première souche française de Cl. bot. Bull. Soc. Vét. Prat. Fr., 33, 1-11.

Prévot, A.-R. et al. 1949. Bull. Soc. Vét. Prat. Fr., 33, 225-

Prévot, A.-R., Huet, M. & Tardieux, P. 1950. Étude de vingt-cinq foyers récents de botulisme animal. Bull. Acad. Vét. Fr., 23, 401-487.

Prévot, A.-R. et al. 1950. Ann. Inst. Past., 78, 274.

Prévot, A.-R. & Brygoo, E.R. 1950. Recherches sur la toxine, l'anatoxine et l'antitoxine botuliques D. Ann. Inst. Past., 79, 1-13.

Prévot, A.-R. & Huet, M. 1951. Existence en France du botulisme humain d'origine piscicaire et de Cl.bot. D. Bull. Acad. nat. Méd., 135, 432-434.

Prévot, A.-R., Huet, M. & Thévenard, A. 1952. Second cas français de botulisme humain d'origine pisciaire. Bull. Acad. nat. Méd., 136, 323.

Prévot, A.-R., Loiseau, J. & Thévenard, A. 1952. Nouveaux cas de botulisme humain d'origine pisciaire. Résultat du traitement par l'anatoxine B. Bull. Acad. nat. Méd., 136, 663.

Prévot, A.-R. & Thouvenot, H. 1952. Ann. Inst. Past., 83, 180-

Prévot, A.-R. & Thouvenot, H. 1952. Nouvelles recherches sur le phénomène d'entrainement des anaérobies a la thermorésistance. Ann. Inst. Past., 82, 497-499.

Prévot, A.-R. 1953 a. Les problèmes du botulisme. Conférence faite à l'Université de Liège.

Prévot, A.-R. 1953 b. Intern. Bull. Bact. Nom. a. Taxon., 3, 120-

Prévot, A.-R. 1953 c. Unification de la nomenclature des types toxinogéniques de Cl.bot. Bull. Acad. Vét. B., 26, 421-422.

Prévot, A.-R. & Brygoo, E.R. 1953 a. Recherches sur les communautés antigéniques entre les cinq toxines botuliques. Ann. Inst. Past., 84, 1037-

Prévot, A.-R. & Brygoo, E.R. 1953 b. Ann. Inst. Past., 85, 255-

Prévot, A.-R. & Brygoo, E.R. 1953 c. Nouvelles recherches sur le botulisme et ses cinq types toxiniques. Ann. Inst. Past., 85, 544-575.

Prévot, A.-R., Brygoo, E.R. & Sillioc, R. 1953. Action of cortisone on the immunization of rabbits by botulinum B. toxin. Ann Inst. Past., 85, 255-257.

Prévot, A.-R., Sillioc, R. & Quentin, M. 1953. Existence en France du botulisme bovine de type C. Bull. Acad. Vet. Fr., 26, 73-78.

Prévot, A.-R., Sillioc, R. & Cay, H. 1954. Étude d'un foyer de botulisme équin de type C. Rec. Méd. Vét., 130, 353-355.

Prévot, A.-R. & Sillioc, R. 1955. Un enigme biologique: Chat et botulisme. Ann. Inst. Past., 89, 354-357.

Prévot, A.-R., Sillioc, R. & Prouté, J. 1955. Étude d'un foyer de botulisme bovin dú a Cl.bot. C. Ann. Inst. Past., 88, 513-515.

Prévot, A.-R., Terrasse, J., Daumail, J., Cavaroc, M., Riol, J. & Sillioc, R. 1955. Existence en France du botulisme humain de type C. Bull. Acad. Méd. (Paris), 139, 355-358.

Prévot, A.-R., Collin, P. & Sillioc, R. 1956. Étude sur le botulisme en France. Rec. Méd. Vét., 132, 188-193.

Prévot, A.-R. & Sillioc, R. 1957. Nouvelles recherches sur le botulisme du vison et du furet et la vaccination antibiot. de ces mustelides. Bull. Acad. Vét., 30, 93-102.

Prévot, A.-R., Raynaud, M., Turpin, A. & Sillioc, R. 1958. Vaccination antibotulique du vison par injection unique d'anatoxine concentrée adsorbée. Acad. Sci. Compt. Rend., 246, 1632-1633

Prévot, A.-R. & Sillioc, R. 1958. Recherches sur l'immunisation anti-botulique C β du lapin par injection unique d'anatoxine concentrée adsorbée. Ann. Inst. Past., 95, 208-211.

Prévot, A.-R., Montloin, R.J. & Sillioc, R. 1959. Nouvelles recherches sur la vaccination antibotulique du vison. Acad. Vét. Fr. B., 32, 147-152.

Proctor, B.E. & O'Meara, J.P. 1951. Food processing with ionising radiations. Food Techn., 5, 376.

Putnam, F.W., Lamanna, C. & Sharp, D.G. 1946. Molecular weight and homogenity of crystalline botulinus A toxin. J. Biol. Chem., 165, 735-736.

Putnam, F.W., Lamanna, C. & Sharp, D.G. 1948. Physico-chemical properties of crystalline Cl.bot. type A toxin. J. Biol. Chem., 176, 401-412.

Quortrup, E.R. & Holt, A.L. 1940. Case report on botulism type C in mink. J. Am. Vet. Med. Ass., 97, 167-168.

Quortrup, E.R. & Holt, A.L. 1940. Detection of potential botulinus-toxin producing areas in western duck marshes with suggestions for control. J. Bact., 41, 363-372.

Quortrup, E.R. & Sudheimer, R.L. 1943. Some ecological relations of P. aeruginosa to Cl.bot. type C. J. Bact., 45, 551-

Quortrup, E.R. & Gorham, J.R. 1949. Susceptibility of furbearing animals to the toxins of Cl.bot. types A, B, C and E. Am. J. Vet. Res., 10, 268-271.

Rauchbach, C. 1956. Lo botulisme de l'homme et des animaux. (In Rumanian) Prob. Zooteh. Vet., 3, 26-32.

Raynaud, M. et al. 1949. Extraction des toxins botuliniques a partir des corps microbiens. Ann. Inst. Past., 77, 316-321.

Raynaud, M. et al. 1951. C.R. Acad. Sci., 233, 586.

Raynaud, M., Turpin, A. Mangalo, R., Bizzini, B. & Péry, R. 1955. Growth and toxinogenesis. II. Ann. Inst. Past., 88, 24-35.

Reames, H.R., Kadull, P.J., Housewright, R.D. & Wilson, J.D. 1947. Studies on botulinum toxoids types A and B. III. Immunization of man. J. Immunol., 55, 309-324.

Reed, J.M., Bohrer, C.W. & Cameron, E.J. 1951. Spore destruction rate studies on organisms of significance in the processing of canned foods. Food Res., 16, 303-408.

Reynolds, H. & Lichtenstein, H. 1950. Effect of certain growth factors on the heat resistance of anaerobic spores. Bacteriological Proceedings, 50, 28.

Rice, C.E. 1947. A prelim. study of the antigenic activity of mixtures of Cl.bot. toxoid types A and B. Canad. J. Res.Sect. E., 25, 181-187.

Rice, C.E., Pallister, E.F., Smith, L.C. & Reed, G.B. 1947 a. Cl.bot. type A toxoid. Canad. J. Res. Sect. E., 25, 167-174.

Rice, C.E., Smith, L.C., Pallister, E.F. & Reed, G.B. 1947 b. Cl.bot. type B toxoid. Canad. J. Res. Sect. E., 25, 175-180.

Rieman, H. 1953. Germination of anaerobic spores. VI Congr. Intern. di microbiologia, Roma, 4, 150-155.

Robinson, R.H.M. et al. 1949. A bact. study of dried whale meat with particular ref. to the presence of Cl. J. Hyg., 47, 236-243.

Rochmann, R. 1956. Feed quality and botulism. Norsk Peladyrbl., 30, 201-202.

Roemmele, 1956. Botulismusvergiftung durch rohe Mettwurst? Arch. Lebensm. Hyg., 7, 97-100.

Rose, A.L. 1934. Bot. bei Schafen und Rindern in Westaustralien. Austr. Vet. J., 10, 175.

Roth, N.G. & Halvorson, H.O. 1952. The effect of oxidative rancidity in unsaturated fatty acids on the germination of bacterial spores. J. Bact., 63, 429-435.

Rycaj, T. 1956. The testing of food for botulinic type A toxin with the aid of the specific haemagglutination method. Acad. Polon. Sci. B. Cl. II, 4, 317-320.

Rydenstam, B.G. 2 fall av botulism. To be published in Nordisk Medicin 1960-1961.

Sakaguchi, G. et al. 1954. Outbreak of type E botulism in Akita Prefecture due to gilthead-isuski. Jap.J.M.Sc. & Biol., 7, 539-545.

Sakaguchi, G. & Tohyama, Y. 1955. Toxin production of Cl.bot. type E. Jap.J.M.Sc. & Biol., 8, 247-262.

Saleh, M.A. 1955. Studies on the growth and toxin production of Cl.bot. in a precooked frozen food. Inhibition by lactic acid bacteria. Diss. Abs., 15, 793-794.

Saleh, M.A. & Ordal, Z.J. 1955. Studies on the growth and toxine production of Cl. bot. in a precooked frozen food. Food Res., 20, 332-350.

Sapinski, W. 1959. A case of bot. in a 9-year old boy. Pediat. Pol., 34, 1561-1562.

Schantz, E.J. & Spero, L. 1957. The reaction of botulinum toxin type A with ketene.
J. Am. Chem. Soc., 79, 1623-1625.

Scheibner, G. 1955. Der Einfluss von Ultraschallwellen auf das Botulinustoxin.
Tierärztl. Umschau, 10, 364-366.

Scheibner, G. 1955. Zum Botulismus beim Nerz in Deutschland.
Mh. Tierheilk., 7, 195-

Scheibner, G. 1955. Die Empfänglichkeit des Schweines für Bot. toxin der Typen A-E.
Dtsch. Tierärztl. Wschr., 62, 355-358.

Scheid, G. 1948. Schinken und Rauchfleisch als Infektionsquelle f. Botulismus.
Med. Mon.-Schr., 2, 312-314.

Schindler, R. & Vocke, G. 1956. Botulismus und Staupe der Nerze und Vorschläge zu ihrer Bekämpfung.
Deut. Pelztierzüchter, 30, 41-45.

Schmidt, F. 1958. Merkblatt über Staupe und Botulismus und ihre Bekämpfung /bei Nerz/.
Deut. Pelztierzüchter, 32, 121-124.

Schmidt, C.F. & Nank, W.K. 1960. Radiation sterilisation of food. I. Procedures for the evaluation of the radiation resistance of spores of Cl.bot. in food products.
Food Res., 25, 321-327.

Schneider, H.J. & Fisk, R. 1939. Botulism: Demonstration of toxin in blood and tissue.
J.A.M.A., 113, 2299-2300.

Schoenholz, P. & Meyer, K.F. 1922. The occurrence of spores of B. bot. in the Hawaiian Islands and China.
J. Infect. Dis., 31, 610-613.

Schoenholz, P. & Meyer, K.F. 1924. Effect of direct sunlight, difuse daylight and heat on potency of Bot. toxin in culture mediums and vegetable products. XXIV.
J. Infect. Dis., 35, 381-389.

Schoop, G. 1954. Untersuchungen über den Botulismus des Pferdes.
Deut. Tierärztl. Wchnschr., 61, 228-231.

Scott, W.J. & Stewart, D.F. 1944. The influence of dissolved tin on the growth of Cl.bot. in canned vegetables.
J. Coun. Sci. Ind. Res. Aust., 17, 16-22.

Scott, W.J. 1950. Thermal destruction of type A Cl.bot. toxin. The nature of the protective substances in canned vegetables.
Austral. J. Appl. Sci., 1, 200-207.

Scott, W.J. & Stewart, D.F. 1950. The thermal destruction of Cl.bot. toxin in canned vegetables.
Austral. J. Appl. Sci., 1, 188-199.

Scott, W.J. 1955. Factors in canned ham controlling Cl.bot. and Staph.aureus.
Ann. Inst. Past., 7, 68-73.

Seddon, H.R. 1922. Bulbar paralysis in cattle due to the action of a toxigenic bacillus with a discussion on the relation of the condition to forage poisoning (Botulism)
J. Comp. Path. & Therap., 35. 147-190.

Seeber, F. 1956. Botulismus als Berufs-Krankheit?
Zeitschr. Ärztliche Fortbildung, 50, 731-733.

Segalove, M. & Dack, G.M. 1951. Growth of bacteria associated with food poisoning experimentally inoculated into dehydrated meat.
Food Res., 16, 118-125.

Shaw, R.M. & Simpson, G.S. 1936. Cl.bot. type C in relation to duck sickness in the province of Alberta.
J. Bact., 32, 79-88.

Shewan, J.M. & Liston, J. 1955. A review of food poisoning caused by fish & fishery products.
J. Appl. Bact., 18, 522-534.

Shupiatskii, M.G. 1959. Unusual case of botulism.
Voen. Med. Zh., no. 4, 25-26.

Shvedov, L. . 1960. Observations on the production of toxins in the gastro intestinal tract of animals infected orally with a culture of the pathogen bot. type A.
Zh. Mikrobiol., 31, 105-111.

Skulberg, A. 1955. Toxicological and bacteriological investigations in connection with an outbreak of botulism. (In Norw.)
Nord. Vet. Med. 7, 207-220.

Skulberg, A. 1957 a. Botulisme. 4 recent outbreaks of human botulism in Norway. (In Norw.)
Nord. Hyg. Ti., 18, 263-267.

Skulberg, A. 1957 b. Botulisme hos mink. (Botulism in Minks) Medlemsblad Norske Vet.for., 9, 41-49.

Skulberg, A. 1958 a. A case of fish-borne type B botulism. Nord. Hyg. Tidskrift, 39, 133-136.

Skulberg, A. 1958 b. Praktiske problemer i forbindelse med botulisme hos dyr. (Practical Problems In Connection With Botulism In Animals) Medlemsblad Norske Vet.for., 10, 277-282.

Skulberg, A. & Coleby, B. 1960. The inactivation of the toxins of Cl.bot. types A and B, by 4 MeV electrons. Risö Report no. 1[illegible], p. 59-60.

Skulberg, A. Human botulism in Norway. Short summary of outbreaks. To be publ. in Nord.Vet.med.1960-61.

Slocum, G.G., Welch, H. & Hunter, A.C. 1941. An outbreak of botulism caused by home canned tomatoes. Food Res., 6, 179-187.

Smith, L.D.S. 1955. Introduction to the pathogenic anaerobes. Univ. of Chicago Press, Chicago, p. 108-133.

Smith, J. 1956. Pathogenic organisms in dairy products. J. Soc. Dairy Techn., 9, 173-180.

Snipe, P.T. & Sommer, H. 1928. Studies on botulinus toxin. III. Acid precipitation of botulinus toxin. J. Infect. Dis., 43, 152-160.

Solem, J.H. & Skulberg, A. 1957. Botulisme - serologisk verifisert. Ti. Norske Lägefor., 77, 210-213.

Sommer, H. 1937. Purification of botulinus toxin. Proc. Soc. Exptl. Biol. Med., 35, 520-521.

Spero, L. 1958. The alkaline inactivation of botulinum toxin. Arch. Biochem. & Biophys., 73, 484-491.

Starin, W.A. 1924. Pure cultures of Cl. bot. from single cells. J. Infect. Dis., 34, 148-158.

Starin, W.A. & Dack, G.M. 1924. Complement - fixation studies on Cl.bot. J. Infect. Dis., 34, 137-147.

Starin, W.A. & Dack, G.M. 1925. Pathogenicity of Cl.bot. J. Infect. Dis., 36, 381-412.

Starin, W.A. 1926. Viability and toxicogenic power of small numbers of spores of Cl.bot. subjected to different temperatures. J. Infect. Dis., 38, 101-105.

Starin, W.A. 1926. Relation of incubation temp. to viability, rate of growth and toxin production of Cl.bot. in different vegetables. J. Infect. Dis., 38, 106-

Staskiewicz, G. 1951. New data on botulismus. (In Polish.) Med. Weter., 7, 181-184.

Steinke, P.K.W. & Foster, E.M. 1950. Effect of sausage constituents on growth of Cl. bot. in liver sausage packed in saran plastic casings. Bact. Proceedings, 50, 29.

Steinke, P.K.W. & Foster, E.M. 1951. Botulinum toxin formation in liver sausage. Food Res., 16, 477-484.

Sterne, M. & Mason, J.H. 1938. The immunization of guinea pigs against the toxins of Cl.bot. types C and D. J. S. African Vet. Med. Ass., 9, 71-73.

Sterne, M. & Wentzel, L.M. 1950. A new method for the large-scale production of high titre botulinum formol-toxoid types C and D. J. Immunol., 65, 175-183.

Sterne, M. & Wentzel, L.M. 1949. Botulism in animals in South Africa. Rept. XIV Intern. Vet. Congr. (London) no. 3, p. 329-331.

Sterne, M. 1954. Hemagglutination by Cl. bot. type D. Science, 119, 440-441.

Stevenson, J.W., Nelson, V.A. & Reed, G.B. 1947. Preparation of Cl. parabot. toxins. Canad. J. Res. E., 25, 14-24.

Stevenson, J.W. & Girvin, G.T. 1953. The effect of Bot. toxin on the bactericidal acetylation of cholin. VI Int. Congr. Microbiol. (Rom), IV, 133-134.

Stover, J.H., Jr., Fingerman, M. & Forester, R.H. 1953. Botulinus toxin and the motor end-plate. Proc. Soc. Exptl. Biol. Med., 84, 146-147

Straka, R.P. & James, L.H. 1935. Further studies on frozen vegetables. /Cl.bot./ J. Bact. Am., 29, 313-322.

Ström, A. 1934. Skinkeforgiftning fremkalt av Bacillus botulinus. (Pork Poisoning Caused By Bacillus Botulinus) Ti. Norske Lägeforen., 54, 633-636.

Stumbo, C.R., Murphy, J.R. & Cochran, J. 1950. Nature of thermal death time curves for P.A. 3679 and Cl.bot. Food Techn., 4, 321-326.

Sugiyama, H. & Dack, G.M. 1949. Some studies on the heat resistance of spores of Cl.bot. Conf. Res. Proc., 74-79.

Sugiyama, H. 1951. Studies on factors affecting the heat resistance of spores of Cl.bot. J. Bact., 62, 81-96.

Sugiyama, H.I. 1952. Effect of fatty acids on the heat resistance of Cl.bot. spores. Bact. Rev., 16, 125-126.

Sutherland, D.N. 1955. Botulism in farm animals. Queensland Agr. J., 81, 97-102.

Sutherland, H.P. 1960. Report of a case of bot. poisoning. J.A.M.A., 172, 1266-1268.

Swab, C. & Gerald, H. 1933. Augenstörungen bei Bot. Brit. J. Ophtalm., 17, 129.

Tanner, F.W. & MacCrea, F.D. 1923. Cl.bot., resistance of spores to moist heat. J. Bact., 8, 269-275.

Tanner, F.W. & Evans, F.L. 1934. Effect of NaCl in meat curing solutions on anaerobic bacteria. Z.bl. Bakt. Abt. 2, Orig., 91, 44-54.

Tanner, F.W. & Oglesby, E.W. 1936. Influence of temperature on growth and toxin production by Cl.bot. Food Res., 1, 401-494.

Tanner, F.W., Beamer, P.R. & Rickher, C.J. 1940. Further studies on development of Cl.bot. in refrigerated foods. Food Res., 5, 323-333.

Tanner, F.W. 1944. Food-borne infections and intoxications. /Statistics not in 2. ed./ p. 390-425. Twin City Printing, Champaign.

Tanner, F.W. & Tanner, L.P. 1953. 2. ed. Food-borne infections and intoxications. p. 496-568. Garrard Press, Champaign.

Tardieux, P., Huet, M. & Björklund, B. 1952. Étude d'infections de catgut par Cl.bot. A. Ann. Inst. Past., 82, 763-

Tarr, H.L.A. 1953. The action of hydroxylamine on bacteria. J. Fish. Res. Bd Can., 10, 69-75.

Theiler, A. 1920. The cause and prevention of Lamziekte. J. Dept. Agric. (S. Africa), 1, 221-244

Theiler, A. & Robinson, E.M. 1927. Der Botulismus der Haustiere. Zschr. f. Infekt. Krkh. d. Haustiere, 31, 165-

Thiebaut, P. et al. 1955. Manifestations oculaires du botulism. Rev. otoneur., Paris, 27, 238-240.

Thomas, C. et al. 1951. Botulism, a complication of Cl.bot. wound infection. Arch. Path., 51, 623-628.

Thygeson, A.S. 1953. Feed poisoning of mink due to Cl.bot. Dansk Pelsdyravl, 16, 114-117.

Todorovitch, K. & Milosavljevitch, A. 1956. First cases of botulism observed at the clinic of infection diseases of Belgrade. (In Serb., with French summary) Glas Srpska Akademija Nauka, Beograd, Odelj Med., 223, no. 11, p. 105-117.

Townsend, C.T. 1929. Comparative study of nontoxic and toxic strains of Cl. parabotulinum. XXXV. J. Infect. Dis., 45, 87-95.

Townsend, C.T., Yee, L. & Mercer, W.A. 1954. Inhibition of the growth of Cl. bot. by acidification. Food Res., 19, 536-542.

Townsend, C.T., Yee, L. & Mercer, W.A. 1954. Inhibition of the growth of Cl. bot. by acidification. Food Res., 19, 636-642.

Treadwell, P.E., Jann, G.J. & Salle, A.J. 1958. Studies on factors affecting the rapid germination of spores of Cl.bot. J. Bact., 76, 549-556.

Tucker, C.E. & Swanson, H. 1939. Outbreak of botulism in Tennessee due to type B Cl.bot. Publ. Health Rep., 54, 1555-1560.

Turner, A.W. & Balos, C.E. 1941. Motile daughter colonies in Cl. oedematiens group and some other Cl.(Cl.bot., Cl. tetani, Cl. septicum)
Austr. J. Exp. Biol. & Med. Sc., 19, 167-174.

Turpin, A., Raynaud, M., Rolyveld, E.H. & Riza, J. 1954. Concentration of different toxins & anatoxins by metaphosphoric acid.
Ann. Inst. Past., 87, 25-37.

Ubertini, B. & Bianchi, E. 1954. Le botulisme des animaux. (Engl. abstr.)
Off. Internatl. des Epiz. B., 42, 514-524.

Underward, E. 1936. Bot. b. Schafen in Westaustralien.
Austr. J. Exp. Biol. & Med. Sc.14,77-91.

Vallée, A., Kreguer, A. & Lyquem, A. 1956. Botulisme canin: première souche isolée en France.
Acad. Vet. de France B., 29, 419-423.

Velikanov, I.N. 1934. Experimental immunization of man against botulism.
Klin. Med., 12, 1802-1806.

Verge, J. 1951. Toxi-infections animales et alimentation. Le botulisme.
Rec. de Méd. Vét., 127, 767-828.

Virat, B., Vallée, A. & Kreguer, A. 1956. A propos du botulismo du mouton: première souche ovine isolée en France.
Acad. Vet. de France B., 29, 425-426.

Wagenaar, R.O. & Dack, G.M. 1953. Some factors influencing the growth and toxin production of Cl.bot. experimentally inoculated into surface ripened cheese.
J. Dairy Sci., 36, 563-564.

Wagonnar, R.O., Dack, G.M. & Mayer, D.P. 1953. Studies on mink food experimentally inoculated with toxin-free spores of Cl.bot. types A, B, C and E.
Am. J. Vet. Res., 14, 479-483.

Wagenaar, R.O. & Dack, G.M. 1954 a. Further studies on effect of experimentally inoculated canned bread with spores of Cl.bot.
Food Res., 19, 521-529.

Wagenaar, R.O. & Dack, G.M. 1954 b. The effect of emulsifier on growth and toxin production of Cl.bot. experimentally inoculated into surface-ripened cheese.
J. Dairy Sci., 37, 640.

Wagenaar, R.O. & Dack, G.M. 1955. Studies on canned cheese spread experimentally inoculated with spores of Cl.bot.
Food Res., 20, 144-148.

Wagenaar, R.O. & Dack, G.M. 1956. Effect in surface-ripened cheese of irradiation on spores and toxine of Cl.bot. types A and B.
Food Res., 21, 226-234.

Wagenaar, R.O. & Dack, G.M. 1958. Factors influencing growth and toxin production in cheese inoculated with spores of Cl. bot. types A and B. Studies with surface-ripened cheese.
J. Dairy Sci., 41, 1182-1200.

Wagenaar, R.O., Dack, G.M. & Murrell, C.B. 1959. Studies on purified type A Cl. bot. toxin subjected to ultracentrifugation and irradiation.
Food Res., 24, 57-61.

Wagenaar, R.O. & Dack, G.M. 1960. Studies on the inactivation of type A Cl.bot. toxin by irradiation with cobalt 60.
Food Res., 25, 279-284.

Wagner, E. 1924. Biochemical activities of B. botulinus, type C and B. parabot. "Seddon." XXIII.
J. Infect. Dis., 35, 353-360.

Wagman, J. & Bateman, J.B. 1951. The behavior of the botulinus toxins in the ultracentrifuge.
Arch. Biochem. Biophys., 31, 424-430.

Wagman, J. & Bateman, J.B. 1953. Botulinus type A toxin: Properties of a toxic dissociation product.
Arch. Biochem., 45, 375-383.

Wagman, J. 1954. Isolation and sedimentation study of low molecular weight forms of type A botul. toxin.
Arch. Biochem., 50, 104-112.

Wasmuth, W. 1948. Über Botulismus.
Deutsche Med. Wchnschr., 73, 636-

Watson, W.E. 1939. Treatment of botulism.
Northwest Med., 38, 382-387.

Weil. L. et al. 1957. Photoxidation of crystalline Cl. bot. type A toxin in the presence of methylene blue.
Arch. Biochem. Biophys., 68, 308-313.

Wentzel, L.M., Sterne, M. & Polson, A. 1950. High toxicity of pure botulinum type D toxin.
Nature, 166, 739-740.

Wheeler, M.W. & Humphreys, E.M. 1924. Isolation of B. botul., type B, from feces by use of blood agar plates in anaerobic jar.
J. Infect. Dis., 35, 305-

White, P.G. & Appleton, G.S. 1957. Field evaluation of bot. toxoid. /Mink./
Natl. Fur News, 29, no. 2, p. 10 & 29.

Willems, R. 1954. Le botulisme du cheval.
Off. Internatl. des Epiz. B., 42, 482-495.

Williams, O.B. & Rood, J.M. 1942. The significance of the incubation temperature of recovery cultures in determining spore resistance to heat.
J. Infect. Dis., 71, 225-227.

Williams, O.B. & Blair, E.B. 1950. Spore formation in synthetic media by Cl. bot.
Bacteriol. Proceed., 50, 62-63.

Williams, O.B. & Fleming, T.C. 1952. Subtilin and the spores of Cl.bot.
Antibiotics & Chemotherapy, 2, 75-78.

Williams, O.B. & Purnell, H.G. 1953. Spore germination growth and spore formation by Cl.bot. in relation to the water content of the substrate.
Food Res., 18, 35-39.

Williams, N.J. & Kempe, L.L. 1957. The effect of Catalase on the lethality of cobalt ^{60} gamma radiation for certain anaerobic bacterial spores.
Appl. Microbiol., 5, 366-368.

Wood, A.J. 1956. Food poisoning of mink. Botulism.
Fur Trade J., 33, no. 8, p. 4-5 & 7.

Wright, G.P. 1955 a. Neurotoxins of Cl. bot. and Cl. tetani.
Pharmacol. Rev., 7, 413-465.

Wright, G.P. 1955 b. Botulinum and tetanus toxins. Mechanisms of microbial pathogenicity.
5th Symp. Soc. Gen. Microbiol., 5, 78-102.

Wright, G.G. et al. 1960. Studies on immunity to toxins of Cl.bot. V. Detoxification of purified type A and B toxins and the antigenicity of univalente and bivalente aluminum phosphate absorbed toxoids.
J. Immun., 84, 304-

Wynne, E.S. 1948. Physiological studies on spore formation in Cl.bot.
J. Infect. Dis., 83, 243-249.

Wynne, E.S. & Foster, J.W. 1948. Physiological studies on spore germination with special reference to Clostridium botulinum.
J. Bact., 55, 61-68 & 69-73.

Wynne, E.S. 1952. Some physiological aspects of bacterial spore formation and spore germination. Symposium on the biology of bacterial spores.
Part III. Bact. Rev., 16, 101-110.

Wynne, E.S., Mehl, D.A. & Schmieding, W.R. 1954. Germination of clostridium spores in buffered glucose.
J. Bacteriol., 67, 435-437.

Wynne, E.S., Smieding, W.H. & Daye, G.T., Jr. 1955. A simplified medium for counting Clostr. spores.
Food Res., 20, 9-12.

York, G.K. & Vaughn, R.M. 1955. Resistence of Cl. parabotulinum to sorbic acid.
Food Res., 20, 60-65.

Zaun, W. 1951. Lebensmittelvergiftungen und andere Intoxikationen durch das Cl. bot.
Kasuistik für die Jahre 1935-50. 43 pp. Giessen.

Zeller, M. 1959. Ein Beitrag zur Botulismusdiagnose.
Arch. Lebensm. Hyg., 10, 265-268.

Zicsko, R. 1951. Botulismus.
Deut. Pelztierzüchter, 25, 225-229.

Zubkova, V.L. 1958. Botulism in a 10 year old girl.
Pediatriia (Moskva), 41, no. 5, 78-79.

Özgen, H. & Özcan, C. 1954. Turkiye sigirlarinda d tipindon ileri gelen botulismus enfeksiyonunun mecudiyeti.
Ankara U. Vet. Fakül. Dergisi, 1, no. 3/4, p. 1-28.

Göteborg den 4 novembor 1960
1675/60-Hg